J. Physiol. (1954) 126, 524–562

CHOLINERGIC AND INHIBITORY SYNAPSES IN A PATHWAY FROM MOTOR-AXON COLLATERALS TO MOTONEURONES

By J. C. ECCLES, P. FATT and K. KOKETSU

Department of Physiology, The Australian National University, Canberra, Australia

(*Received* 22 *June* 1954)

Although a central inhibitory action of antidromic impulses in motor-nerve fibres had been postulated or sought for on several occasions (Brown, 1914; Eccles & Sherrington, 1931; Forbes, Smith, Lambert, Caveness & Derbyshire, 1933), it was first demonstrated by the refined experiments of Renshaw (1941), and has since been investigated by Lloyd (1946, 1951*b*). There is general agreement that the antidromic inhibitory action resembles direct inhibition in the brevity of its latent period, but differs in its relatively long duration, 40–50 msec, as against about 15 msec for direct inhibition. There is also general agreement that the antidromic inhibitory action is exerted preponderantly on motoneurones, the somas of which are located close to the somas of those being activated, while functional relationship is not an essential factor.

The classical neurohistological investigations employing the Golgi technique revealed that the axons of nerve cells often gave off collateral branches soon after their origin, and there are extensive accounts of axon collaterals in the older literature (Kölliker, 1891; Lenhossek, 1893; Cajal, 1909). However, the precise mode of termination of these axon collaterals has remained obscure. Most of the axon collaterals of motoneurones are shown terminating in the ventral horn, but whether they establish synaptic connexions with motoneurones or with interneurones is an open question. Nevertheless, neurophysiologists have not hesitated to postulate functional connexions by which these axon collaterals would exert inhibitory or excitatory actions on motoneurones (Brown, 1914; Gesell, 1940; Renshaw, 1941; Holmgren & Merton, 1954). In particular, Renshaw (1941, Fig. 6D) depicted a motor-axon collateral with postulated connexions either directly to a neighbouring motoneurone, or indirectly through an interneurone, but he regarded such explanations of the antidromic inhibitory action as purely speculative. As an alternative explanation he proposed that the inhibition was caused by the polarizing effects of

electric currents generated by the responses of motoneurones. This explanation has been further developed by Brooks, Downman & Eccles (1950) and by Lloyd (1951*b*), who have shown that the positive after-potential generated by the antidromic invasion of a motoneurone would produce currents which would depress adjacent motoneurones for approximately the duration of the observed antidromic inhibition. However, it must be questioned if such currents are of sufficient intensity to cause the observed inhibition (cf. Renshaw, 1941).

In a later investigation Renshaw (1946) discovered that interneurones in the ventral horn discharged repetitively at high frequency in response to an antidromic volley in motor axons. He did not attempt to relate rigorously the operation of these interneurones to the above-described inhibition, but considered that the establishment of a causal relationship would have to await anatomical evidence. The relationship is established in the present paper by recording electric potential changes in the cell bodies of individual motoneurones and by demonstrating the parallel effects of different chemical substances on the potential changes in the motoneurones on the one hand and on the discharges of the interneurones on the other.

Questions of some general interest are raised by this investigation. It is shown that, in the inhibition of neighbouring motoneurones, electric fields need not be postulated to play a significant part, but instead all intercellular effects can be accounted for satisfactorily by synaptic processes which have specific chemical sensitivity. With this case eliminated as a possible instance of the interaction of neurones by electric fields, the probability that such fields might be important in the functioning of the central nervous system is lessened. Another point which is raised concerns the specific type of transmission occurring in the synapses of this pathway. Strong evidence is produced indicating that at the synapses formed by the collaterals of motor axons on the interneurones transmission is mediated by acetylcholine.

A preliminary report of this investigation has been published (Eccles, Fatt & Koketsu, 1953).

METHOD

The experiments have been performed on the sixth and seventh lumbar segments of the spinal cord of cats under light pentobarbital anaesthesia.

The microelectrode techniques for the intracellular recording of electric potentials from motoneurones and for the exploration of extracellular potential fields among groups of neurones have been described in recent papers (Brock, Coombs & Eccles, 1952; Eccles, Fatt, Landgren & Winsbury, 1954).

The technique for administering solutions of drugs to the lower lumbar region of the spinal cord by injection into its arterial blood supply resembles that used by Holmstedt & Skoglund (1953). Injections were made into the lower part of the aorta. All the main vessels below the renal arteries were ligated, except the lumbar arteries which supply the spinal cord.

RESULTS

A. *Antidromic inhibitory post-synaptic potential*

When the central end of the severed motor nerve to a muscle is stimulated and the appropriate dorsal roots are cut, only the antidromic volley in the motor-nerve fibres is able to enter the spinal cord. The most obvious effect of this volley is to generate action potentials in the central parts of those motoneurones whose axons are excited. In addition to this antidromic activation, the volley has another effect on motoneurones. By testing the electric potentials occurring in a number of motoneurones, it is revealed that this volley increases the membrane potential in a large proportion of those located at the segmental levels at which the antidromic volley enters the spinal cord. For example, in Fig. 1A–F an antidromic volley in the biceps-semitendinosus motor axons caused a microelectrode placed within motoneurones belonging to several different muscles to become more negative relative to an indifferent lead. When it was extracellular, virtually no potential change of this form was observed (cf. Figs. 1M, N and 3A–D); hence the potential change represents entirely a hyperpolarization of the motoneuronal membrane, and the electrical processes producing it must be intrinsic to this membrane.

When the motoneurone belonged to the muscle whose motor nerve was stimulated, its response to the antidromic invasion would be superimposed on any hyperpolarizing response. However, by combining several procedures it has been possible to discriminate between, on the one hand, the various potential changes evoked in a motoneurone when it was antidromically invaded (Brock, *et al.* 1952, 1953), and, on the other hand, the potential change indentifiable with the hyperpolarizing response described above. For example, in Fig. 2A the stimulus to the motor nerve was adjusted so that it was just-threshold for the motor axon belonging to the motoneurone that was impaled by the microelectrode (cf. also Fig. 1A). About one-third of the stimuli excited this axon (response *a*) and two-thirds failed (response *b*). The difference between responses *a* and *b* may be assumed to give the response which is generated in a motoneurone by an impulse in its axon. The response so calculated is seen in Fig. 2D (continuous line) to have the characteristic features of the after-potentials of a motoneurone. Fig. 2B (response *c*) further shows that, on strengthening the stimulus to maximum for the motor nerve, there was an additional hyperpolarization above that observed for the just-threshold response. As a first approximation it may be assumed that the difference between response *c* and that attributable to antidromic invasion of the motoneurone $(a-b)$, i.e. $(c-(a-b))$, gives the total hyperpolarizing response of the type observed when stimulating motor nerves other than that nerve containing the axon of the cell under observation (dotted line, Fig. 2D). The steep

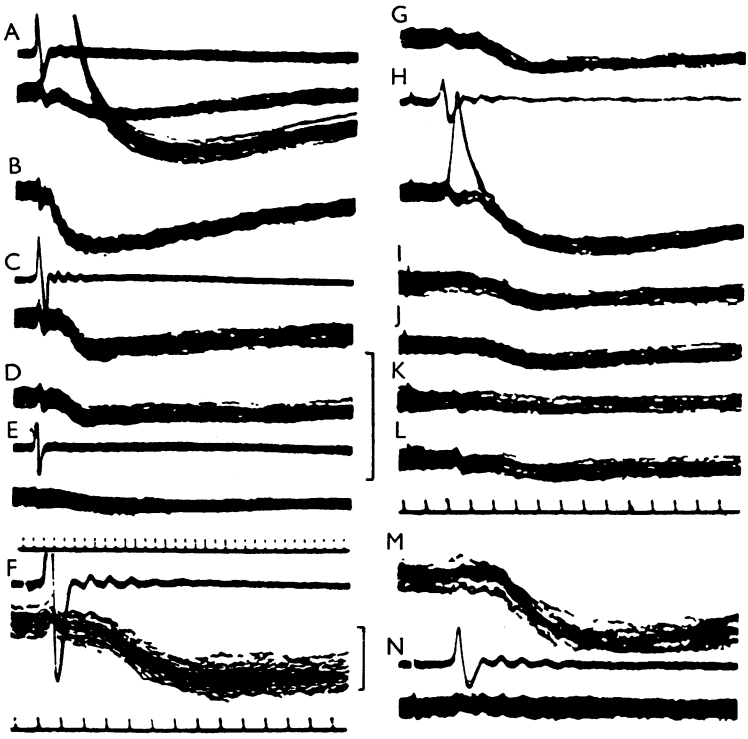

Fig. 1. Potentials generated in a motoneurone by an antidromic volley in motor-nerve fibres and recorded between an intracellular electrode and an indifferent electrode. Downward deflexions signal increasing intracellular negativity. Each record is made by the superposition of about forty faint traces so as to reject random noise as far as possible. This procedure has been adopted for all the illustrated recordings from motoneurones except Fig. 4 D–H and Fig. 17 F. In A, C, E, F, H and N accompanying records show spike potentials recorded simultaneously from the dorso-lateral surface of the spinal cord. Negativity relative to the indifferent electrode is downwards. A to F show responses evoked in six motoneurones of different function by a maximum antidromic volley in biceps-semitendinosus motor axons. The motoneurones are those supplying biceps-semitendinosus, semimembranosus, plantaris, flexor digitorum longus, deep peroneal, and gastrocnemius muscles respectively. Time scale below E gives msec for A, C, D and E; time scale below F gives msec for F. For G–L microelectrode was in a biceps-semitendinosus motoneurone and antidromic volleys were in motor fibres of semimembranosus, biceps-semitendinosus, gastrocnemius, plantaris, flexor digitorum longus and deep peroneal nerves respectively. For M and N the antidromic volley was in gastrocnemius motor fibres and the microelectrode in a flexor digitorum longus motoneurone, M, and immediately outside it, N. Time scale below L gives msec for G–N. In A and H the stimulus applied to the motor nerve was at threshold strength for exciting the axon of the motoneurone under observation. The shorter perpendicular line gives 1 mV potential scale for C, F, M and N; the longer line gives 5 mV for the remaining records.

spike potential curves of the *a* and *c* responses make the calculated curve indeterminate for the first 3 msec.

Since it cannot be assumed that the hyperpolarization is accurately summed with the after-potentials following antidromic invasion of a motoneurone, it was fortunate that in some motoneurones the antidromic impulse was blocked downstream from the motoneurone, probably at the medullated-non-medullated junction (cf. Brock *et al.* 1953). Under such conditions only a small

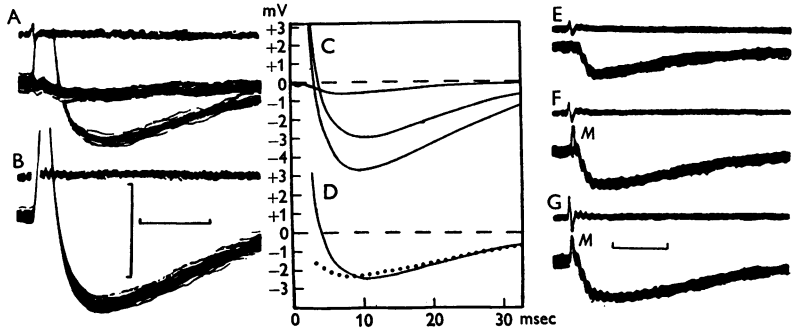

Fig. 2. Motoneurone potentials and accompanying surface potentials evoked and recorded as in Fig. 1. A and B show potentials recorded in a biceps-semitendinosus motoneurone by an antidromic volley set up in biceps-semitendinosus motor fibres (A) by a stimulus which is just-threshold for the axon of the motoneurone and (B) by a maximum stimulus. Plotted responses of A and B are drawn in C and the subtracted curves in D (see text). Zero time signals the arrival of the antidromic volley at the spinal cord. E to G are the responses of a semimembranosus motoneurone to an antidromic volley in semimembranosus axons. For F the stimulus was just-threshold for the axon of that motoneurone, while in E it was below threshold and in G the stimulus was maximum for all motor fibres. Small spike evoked by the antidromic impulse is labelled *M* in F and G. Time scale in 10 msec is drawn for each series; potential scale is 5 mV.

spike with no detectable after-potential was recorded by a microelectrode in the motoneurone whose axon was stimulated. For example, in Fig. 2F a stimulus strength was chosen so that the motor axon was not excited at every trial. The only indication that the axon was excited is the small spike (*M*) that was evoked in about half the superimposed responses. Following this spike there was accurate superimposition of all the responses with no trace of the two distinct types of responses *a* and *b* as seen in Figs. 1A and 2A. Thus in the three records (Fig. 2E, F, G), the hyperpolarization was recorded free from complication by after-potentials. Our many series of observation of this type (cf. Figs. 1H, 3H and I, 4A, 17A) have served to establish that, apart from the response caused by antidromic invasion of a motoneurone, the impulse in a motor axon has no special hyperpolarizing action on the motoneurone to which this axon belongs.

Fig. 1G–L shows typically that a hyperpolarization is usually evoked in

any one motoneurone by antidromic volleys in the motor axons supplying several different muscles of diverse functions. The complete results of our investigations on motoneurones in the seventh lumbar segment have been assembled in Table 1. Usually large hyperpolarizations have been evoked by volleys in the motor nerve containing the axon of the motoneurone being studied, e.g. from semimembranosus to semimembranosus in Table 1. Closely allied groups of motoneurones such as plantaris and gastrocnemius-soleus have exhibited similarity both in respect of the hyperpolarizing actions of antidromic volleys (columns 4 and 5) and the responses of motoneurones (rows 4 and 5). However, reciprocity between the action of the antidromic volley

TABLE 1. Mean values in mV of inhibitory post-synaptic potentials evoked in various types of motoneurones by antidromic volleys in various motor-nerve fibres. The neuronal types are arranged in rows (numbers in brackets giving numbers of the type), the nerve types in columns, both sets having the same identifying symbols: BST = biceps-semitendinosus (excluding anterior biceps); AB = anterior biceps; SM = semimembranosus; GS = gastrocnemius-soleus; P = plantaris; FDL = flexor digitorum longus; DP = deep peroneal; Q = quadriceps; CVR = contralateral L_7 ventral root.

Neuronal type	Nerve volley types								
	BST	AB	SM	GS	P	FDL	DP	Q	CVR
BST (13)	0·55	—	0·32	0·18	0·03	0·03	0·16	0	0
AB (3)	0·15	—	2·37	0·42	0·12	0	0·02	0	0
SM (4)	1·26	—	1·44	0·62	0·28	0·05	0·21	0	0
GS (2)	0·30	—	0	0·50	0·43	0·17	0	0	0
P (5)	0·62	—	0·04	1·31	0·54	0·29	0·08	0	0
FDL (5)	0·52	—	0·15	1·26	0·55	0·18	0	0	0
DP (4)	0·19	—	1·36	0	0	0·09	2·27	0·22	0

and the motoneuronal response was not a general rule, as, for example, may be seen with the gastrocnemius-soleus antidromic volley effectively hyperpolarizing semimembranosus motoneurones, while gastrocnemius-soleus motoneurones were unaffected by a semimembranosus antidromic volley. A failure of reciprocity is also seen in Table 1 with plantaris and biceps-semitendinosus. It is certainly established that the hyperpolarizing action of an antidromic volley in a muscle nerve is exerted on motoneurones of diverse function—flexors or extensors at the various joints of a limb. No meaningful co-ordination pattern can be detected; the only determining factor appearing to be proximity in the spinal cord (cf. Renshaw, 1941). When the antidromic volley entered the spinal cord about one segment more rostral than the motoneurones, as with a quadriceps antidromic volley, Table 1 reveals that the only hyperpolarization that was observed in the motoneurones of the L_7 segment occurred with two deep peroneal motoneurones in the extreme rostral part of L_7 segment. Probably the hyperpolarizing action of an antidromic volley extends along the spinal cord for no more than half a segment from the zone of entry of the volley, while large hyperpolarizing actions are exerted only on motoneurones at the same segmental level. No hyperpolarization has ever

been observed when the antidromic volley was in a contralateral ventral root, i.e. the effect is strictly ipsilateral.

In measuring the latent period of the hyperpolarization it was important to withdraw the microelectrode from the motoneurone and take an extracellular record immediately after the intracellular record. Two such series are shown

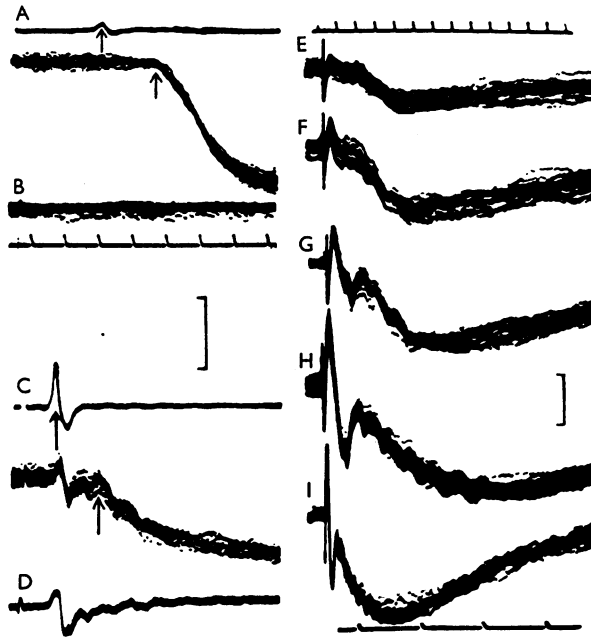

Fig. 3. Motoneurone potentials and accompanying surface potentials evoked and recorded as in Fig. 1. A was obtained with the microelectrode in a deep peroneal motoneurone, and in B after having withdrawn the microelectrode to an extracellular position. The first arrow marks the time of entry of the antidromic volley into the spinal cord, while the second gives the onset of the hyperpolarization. C and D were recorded as in A and B, but from a semimembranosus motoneurone. E–H are from an unidentified motoneurone showing the potentials generated by antidromic volleys of progressively increasing size in the seventh lumbar ventral root, H being maximum. I is the same as H, but at slower sweep speed. Time scale below B is in msec for A–D; above E in msec for E–H, and below I in 10 msec for I. Perpendicular scales give 1 mV for corresponding records.

in Fig. 3 A, B and C, D respectively. In the A, B series the extracellular record shows very little potential change and the onset of the hyperpolarization is easily determinable (second arrow), while the time of entry of the antidromic volley into the spinal cord is given by the crest of the positive (upward) spike as recorded by the surface electrode (first arrow). The latent period as measured between the two arrows was about 1·6 msec. In the C, D series the extracellular record shows an initial series of waves resembling those recorded intra-

cellularly, and the two records must be carefully compared in order to determine where they start to diverge (second arrow). The latent period so measured between the two arrows was 1·25 msec. In our experiments the extreme range of measured latent periods for the hyperpolarization was 1·1–1·8 msec, but probably still longer values would obtain for very small hyperpolarizations.

The duration of the rising phase of the hyperpolarization has varied considerably, from 3 to almost 10 msec, according as the hyperpolarization was small or large respectively. This effect is best seen in series such as Fig. 3E, F, G, H, where the size of the antidromic volley has been progressively increased (cf. also Fig. 2E, F, G). On careful examination of the rising phases of the large hyperpolarizations it is seen to be formed by superposition of a series of wave-like additions at a frequency of about 1000/sec (cf. Figs. 2G, 3H and 4A). Towards and beyond the summit this rhythmic composition gradually became obscured. The origin of this repetitive wave, together with the alteration of latency with response size, will be considered in a later section.

It has been shown that a direct inhibitory volley generates a hyperpolarization of motoneurones that has a latent period sufficiently short and a time course sufficiently long for it to be causally related to the direct inhibition (Eccles, 1953, p. 158; Eccles, Fatt & Landgren, 1954); hence this hyperpolarization has been called the inhibitory post-synaptic potential (IPSP). It remains now to inquire: (i) whether the hyperpolarization that an antidromic volley produces in motoneurones has the latent period and time course that would be expected if it were causally related to the antidromic inhibitory action discovered by Renshaw (1941); and (ii) whether the antidromic hyperpolarization is generated by a process similar to that which generates the IPSP of direct inhibition. The general time course of large antidromic hyperpolarizations as, for example, in Figs. 2G and 3I, corresponds closely to the antidromic inhibitory curves of Renshaw (1941) and Lloyd (1946, 1951*b*) with summits at about 10 msec and total durations usually of about 40–50 msec. Comparison of the respective latent periods is complicated on account of the fairly wide range of synaptic delays for responses of the various motoneurones giving the testing monosynaptic reflex discharge. Renshaw (1941) concluded that inhibition was first induced when an antidromic volley arrived at the motoneurones approximately simultaneously with the afferent impulses that were evoking the testing monosynaptic reflex. Since the longest latent period for the reflex discharge was considerably greater than 1 msec, it may be concluded that the observed latent period for the hyperpolarization (1·1–1·8 msec) is just brief enough to account for the observed latent period of the inhibition. The relative timing is best appreciated if attention is focused on the time of the testing reflex discharge, for inhibition can occur so long as the reflex discharge of longest latency is not antecedent to the onset of the hyperpolarization (Eccles, 1953; Eccles, Fatt & Landgren, 1954).

Various experimental procedures have established that an identical process gives the antidromic hyperpolarization and the other inhibitory post-synaptic potentials. Two simple tests are illustrated in Fig. 4. Exactly as would be expected for an IPSP (Coombs, Eccles & Fatt, 1953), introducing chloride ions into a motoneurone causes the antidromic hyperpolarization (Fig. 4A) to be converted to a depolarization (Fig. 4B), which may become very large. This

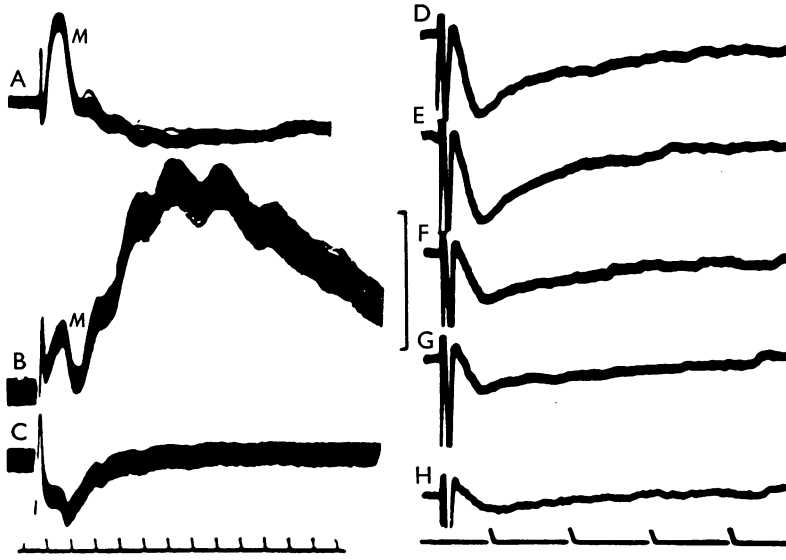

Fig. 4 A to C. Potentials generated in biceps-semitendinosus motoneurone by an antidromic volley in the seventh lumbar ventral root. Note initial *M* spike. Chloride is injected into the motoneurone by passing $2{\cdot}5 \times 10^{-8}$ A for 120 sec through the microelectrode, thereby changing the hyperpolarization of A to the depolarization of B. C shows extracellular potential recorded on withdrawal of microelectrode from the motoneurone. Time is in msec. Potential scale gives 5 mV.

Fig. 4 D to H. The effect of strychnine in depressing a potential which had been previously shortened by the injection of dihydro-β-erythroidine hydrobromide (cf. Fig. 17C, D). Single traces only are recorded. The potentials are generated in a quadriceps motoneurone by an antidromic volley in the sixth lumbar ventral root. 0·1 mg strychnine hydrochloride/kg body weight was injected intravenously between D and E. E to G were recorded at 10, 20 and 30 sec after the injection. Maximum depression was attained at G. H shows the maximum depression after a further 0·1 mg/kg. Time is in 10 msec. Potential scale is the same as for A–C.

large inverted potential shows very well the constituent rhythm on its rising phase and summit. Further evidence relating to the synaptic origin of the antidromic inhibitory potential is given by the depressant action of strychnine. For example, intravenous injection of 0·1 mg strychnine hydrochloride per kg caused the antidromic hyperpolarization rapidly to decrease from the initial size (Fig. 4D) to about half (Fig. 4G), while an additional similar dose caused

a further diminution (Fig. 4H). The same relative diminution is observed with the direct IPSP, and provides a satisfactory explanation of the depression of inhibitory action by strychnine (Bradley, Easton & Eccles, 1953). The identity of the antidromic hyperpolarization and an IPSP is also indicated by the similar changes produced in them when the membrane potential of the motoneurone has been changed over a wide range by extrinsic currents (Coombs *et al.* 1953).

These investigations lead to the conclusion that the antidromic hyperpolarization is produced by ionic movements across the motoneuronal membrane which are identical with those giving the IPSP of direct inhibition. It is probable that strychnine depresses direct inhibition by blocking the postsynaptic receptors for the inhibitory transmitting substance (Bradley *et al.* 1953); hence the similar depressant action of strychnine on antidromic hyperpolarization indicates that it is caused by mediation of the same transmitting substance. It is therefore justifiable to identify the antidromic hyperpolarization as a true IPSP and to attribute it to the action of a synaptic mechanism similar to that giving the direct IPSP. It remains now to determine the pathway by which antidromic impulses activate inhibitory synapses on motoneurones.

B. *Interneuronal discharges*

On account of the paucity of experimental evidence Renshaw (1946) refrained from concluding that the antidromic inhibitory action was mediated by the repetitive interneuronal discharges which he found to be evoked in the ventral horn by a volley in motor axons. Important additional evidence for this causal relationship is provided by the rhythmic steps on the rising phase of large antidromic IPSP's (cf. Figs. 2G, 3H and 4A, B), which suggests that the antidromic IPSP is produced by a repetitive synaptic bombardment. The frequency of this wave is approximately the same as the interneuronal discharges observed by Renshaw. In addition to confirming almost all of Renshaw's findings, a detailed study of the interneuronal discharges has established that these interneurones form a specialized group mediating the inhibitory path from motor axons. They may appropriately be given the distinguishing title of 'Renshaw cells'.

Convergence of motor impulses on to Renshaw cells. Usually antidromic impulses in axons of several motor nerves exert an excitatory action on any one Renshaw cell. For example in Fig. 5A–E maximum volleys in biceps-semitendinosus, gastrocnemius, flexor digitorum longus, plantaris and superficial peroneal nerves evoked respectively 14, 5, 3, 2 and 1 discharges from the same Renshaw cell (note characteristic shape of spike), while in Fig. 5F–H volleys in semimembranosus, tibialis posterior and deep peroneal nerves were ineffective. The slower record (Fig. 5I) shows that the BST volley actually evoked 22 discharges from the Renshaw cell over a total duration of about

40 msec. It has invariably been observed that the frequency of discharge is highest for the first impulses and progressively falls thereafter. Fig. 5K–Q shows the responses of another Renshaw cell to various sizes of an antidromic volley. These sample records illustrate seven distinct gradations of response

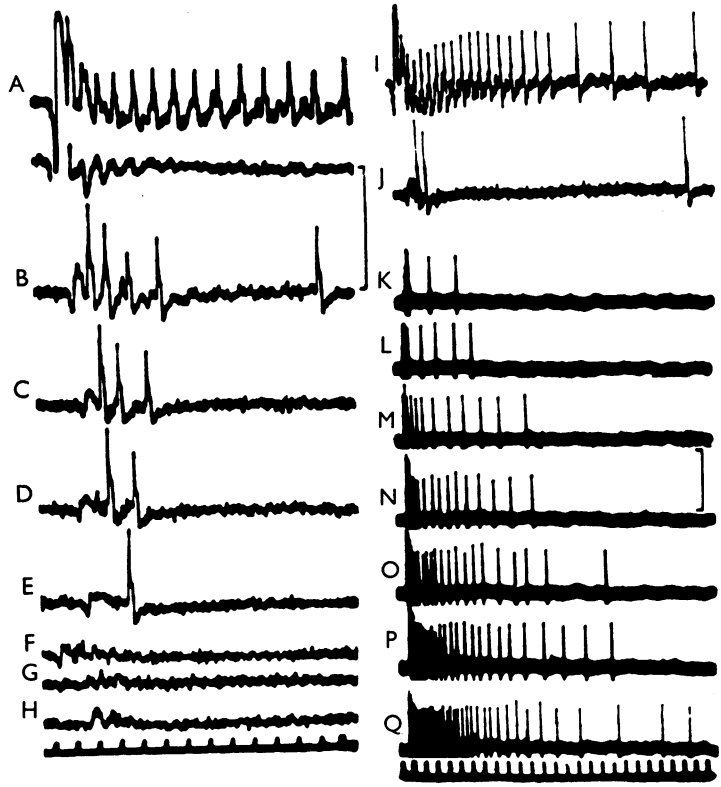

Fig. 5 A to E show discharges of a Renshaw cell generated by antidromic volleys in various motor nerves as described in text. The surface responses are also shown in A with the exception of the initial diphasic spike. F–H show the failure of antidromic volleys in three other motor nerves. Usually the diphasic antidromic response of the motoneurones can be seen preceding the first Renshaw cell spike. Time is in msec. Potential scale gives 1·0 mV. I and J show responses to the same volley as A and D respectively, but at one third the sweep speed. K–Q give specimen records of seven different intensities of response evoked in another Renshaw cell by a progressively increasing antidromic volley. The initial high frequency response cannot be resolved at the sweep speed employed. Time is in 10 msec. Potential scale gives 1·0 mV.

that were revealed by some 90 records with gradually increasing volley size. Several such gradations were always observable with intense Renshaw cell responses. Thus it may be concluded that the Renshaw cell illustrated in Fig. 5A–J was converged upon by impulses in probably as many as ten different axons distributed over at least five motor nerves. It was always

possible to demonstrate the convergence of impulses in several different motor axons upon any Renshaw cell.

Latent period, frequency and duration of discharge. The Renshaw cell discharge illustrated in Fig. 6A is shown at higher amplification and faster speed in Fig. 6E in order to allow accurate measurement of latent period and initial frequency. When measured from the arrival of the antidromic volley at the

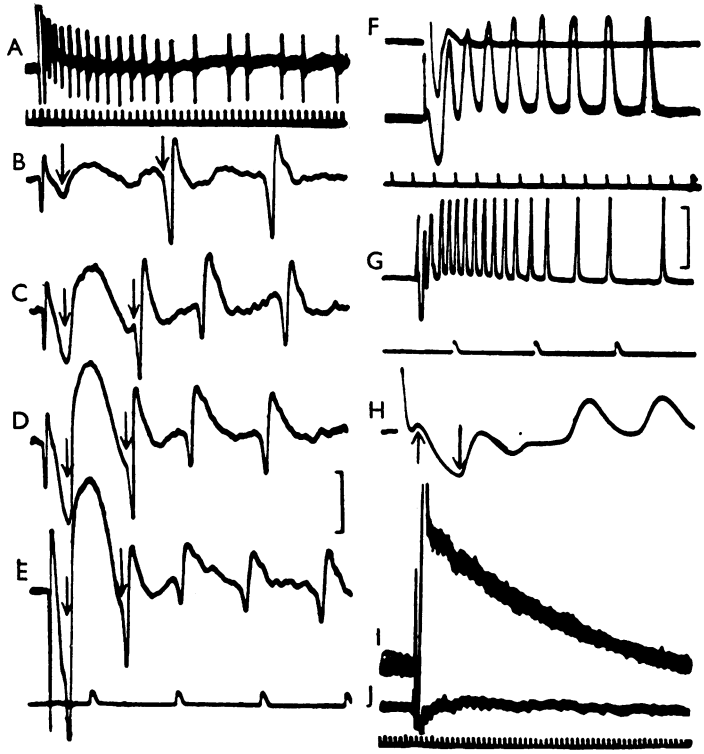

Fig. 6. B–E show responses evoked in a Renshaw cell by antidromic volleys of progressively increasing sizes in the seventh lumbar ventral root and recorded at a fast sweep speed. A is at a much slower speed. Note the diphasic motoneuronal response preceding the first Renshaw cell spike. Latent periods are measured between the two arrows. With A and E the motor axon volley is maximum. Time is in msec. Potential scale gives 1 mV. F–H are the responses evoked in another Renshaw cell by a maximum volley in seventh lumbar ventral root and recorded intracellularly, upward deflexion indicating positivity of microelectrode relative to the indifferent electrode, i.e. it is the reverse of other figures in which the Renshaw cell discharges have been recorded extracellularly. With F the surface response is also shown inverted. Three different sweep speeds are used as shown by the msec time scales for F and H, G scale being as for A. Smaller responses in H are due to progressive failure of cell. Latent period is measured between the two arrows, the initial motoneuronal response appearing inverted. Note that the second of the four Renshaw cell responses is so early that it is greatly reduced in size. Potential scale gives 5 mV. I and J show potentials recorded inside (cf. F–H) and just outside another Renshaw cell at much slower sweep speed (see time scale in msec). Potential scale is the same as for A–E. Further description is given in the text.

spinal cord (first arrow), the latent period of onset of the first discharge was 0·62 msec, while the subsequent intervals between discharges were 0·62, 0·76 and 0·88 msec respectively. Progressive diminution of the antidromic volley size in Fig. 6E–B lengthened both the latent period and the intervals between discharges. The latent period in Fig. 6B was 1·16 msec. Still longer latent periods have been observed with antidromic volleys that just evoked a discharge. For example, in Fig. 5D it was 1·4 msec for the first plantaris response and it was almost 1·9 msec for the single response evoked by the superficial peroneal volley (Fig. 5E), which is one of the longest latent periods that we have observed, and considerably longer than the longest value illustrated by Renshaw (about 1·5 msec). In general, when an antidromic volley evoked a fast repetitive response of Renshaw cells, the latent period of the first response was between 0·6 and 0·7 msec, values which are in precise agreement with those reported by Renshaw (1946). An exceptionally brief latent period of 0·54 msec was observed with a Renshaw cell into which a microelectrode had been inserted (Fig. 6H), an effect which presumably is attributable to the depolarizing influence of the injury. Usually the latent period was so brief that the first Renshaw cell spike arose during the declining phase of the soma spike potential (cf. Figs. 5A, 11F).

Likewise the highest frequencies that we have observed are in good agreement with Renshaw's value of about 1500/sec. It was invariably observed that the shortest interval was between the first two responses, there being a progressive lengthening of interval with each successive response (Figs. 5A, I, 6A, G, 11A, F, 13A, B), so that after ten responses the frequency was always well below 1000/sec. At such high initial frequencies the successive spikes were, as expected, subnormal in size (cf. Figs. 5A, I, 11A, F, 13A, B). Usually the decline in frequency was fairly regular, but there was often wide variation in the intervals between the last few responses, as, for example, may be seen in the last response to the gastrocnemius volley in Fig. 5B occurring after an interval of 7·5 msec, and occasionally intervals longer than 20 msec were observed in later observations on this same Renshaw cell, as is illustrated in Fig. 5J, where a third response occurred more than 30 msec after the first two. There has been great variability in the total duration of the Renshaw cell discharge generated by a single antidromic volley. However, after a powerful excitatory action generating a high-frequency discharge, the discharge usually continued for 30–50 msec, but exceptionally durations in excess of 100 msec have been observed (Fig. 5Q).

Usually a penetrating microelectrode kills a Renshaw cell almost instantaneously, there being a brief high-frequency response at the instant of penetration. Exceptionally it has been possible to record intracellularly from Renshaw cells for a brief period as in Fig. 6F–I. However, there was always evidence of severe injury of such Renshaw cells as revealed both by the very

low voltage of the responses and by their rapid deterioration. Such recording has been of interest solely because it gives the time courses of the background synaptic depolarization and of the spike responses generated thereby. In Fig. 6F, G the spikes are seen to arise from a background depolarization of rather less than 1 mV. However, the intracellular recording was very imperfect as the spike potentials were less than 10 mV. On a few rare occasions the potential wave form illustrated in Fig. 6I has been generated by an antidromic volley immediately after penetration of a Renshaw cell. Apparently, after an initial spike discharge, a large depolarizing potential gradually decays over more than 40 msec. This is precisely the time course of the depolarization that would be expected to evoke the repetitive Renshaw cell discharges with their progressive decline from a high initial frequency.

Anatomical pathway for Renshaw cell activation. It has been suggested by Toennies & Jung (1948), Toennies (1949) and Jung (1953) that Renshaw cells are activated by the reversed synaptic action of antidromic impulses that propagate to the terminals of the dendrites of the motoneurones. On the contrary, Renshaw (1946) suggested as a possibility that the antidromic impulses would invade the motor-axon collaterals and so might excite the Renshaw cells synaptically. He suggested, alternatively, synaptic excitation by 'hypothetical afferent fibres in the ventral roots'. Actually his own observations would exclude this last suggestion, for he observed that the same interneurone could be activated secondarily to a reflex discharge as well as by an antidromic volley, an observation which has repeatedly been confirmed in our experiments. Furthermore, the range of stimulus thresholds of the fibres activating Renshaw cells has conformed precisely with the thresholds for large motor axons (cf. Figs. 5K–Q, 6B–E). No additional excitatory effect on Renshaw cells has been detectable with stimuli strengthened sufficiently to excite the small motor axons.

It is possible to distinguish experimentally between the two remaining alternative mechanisms for activation of Renshaw cells. Normally only a fraction of the dendrites of a population of motoneurones is invaded by an antidromic volley, and this invaded fraction can be greatly increased by the depolarization produced by excitatory synaptic action (Renshaw, 1942, Lloyd, 1943; Brooks & Eccles, 1947; Brock *et al.* 1953). Thus, if Renshaw cells are activated by impulses in the motoneuronal dendrites, facilitation of the antidromic invasion of the dendrites by a suitably timed monosynaptic excitatory volley should effectively increase the Renshaw cell response to a given antidromic volley. On the other hand, the antidromic invasion of motor-axon collaterals should not be appreciably enhanced by a monosynaptic excitatory volley, and hence this volley should cause no modification of the Renshaw cell discharge. It was important to avoid a complication that occurred when the monosynaptic excitatory volley itself caused a discharge

of the Renshaw cell as a consequence of the reflex discharge which it evoked. Fig. 7 illustrates two of the nine series of investigations, all of which showed that facilitation of the antidromic invasion by a monosynaptic excitatory volley (upper graph) had no effect on the activation of Renshaw cells by that antidromic volley (open and filled circles in lower graph). The control observations were provided by the Renshaw cell discharges set up by the antidromic

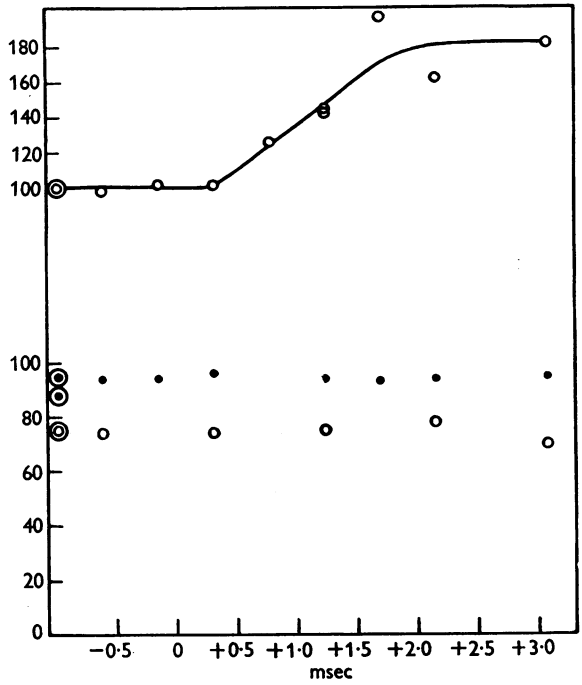

Fig. 7. Abscissae give intervals between the arrival times at the spinal cord of an afferent volley travelling via the dorsal roots from gastrocnemius and biceps-semitendinosus nerves and of an antidromic volley in the seventh lumbar ventral root. The upper curve shows the time course of facilitation of antidromic invasion of motoneuronal somas and dendrites, the ordinates being sizes of the antidromic soma spike potentials as percentages of control size in the absence of a conditioning afferent volley. Each filled and open circle below plots the total number of impulses discharged by a Renshaw cell in response to six separate tests at the plotted interval between arrival of the afferent and the antidromic volleys. Control numbers for antidromic volleys alone are indicated by ringed values. Further description is given in the text.

volley alone and by the antidromic volley when it was too early for its invasion to be facilitated by the monosynaptic excitatory volley (intervals of −0·61 to +0·31 msec in Fig. 7). Each point plotted in Fig. 7 represents the aggregate number of Renshaw cell discharges generated in six successive tests by the antidromic volley either alone or in the indicated time relationship to the monosynaptic excitatory volley.

The uniformly negative results of our experiments lead to the conclusion that Renshaw cells are activated by antidromic impulses spreading up the motor-axon collaterals to terminals that make synaptic contacts with Renshaw cells. It may be assumed that reflexly discharged impulses from motoneurones would also spread along these axon collaterals, hence an explanation is available for the generation of Renshaw cell discharges by afferent volleys entering the spinal cord through dorsal roots. As expected, such volleys in group I afferent fibres are effective only when they evoke a considerable reflex discharge.

Further physiological experiments on Renshaw cells. In his investigation on the interaction of two antidromic volleys, Renshaw (1946) described both summation and inhibition. Summation has been a regular finding in our experiments, and has been demonstrated with two volleys neither of which produced a discharge. The summation interval has been as long as 50 msec. When each volley alone produced a repetitive discharge, the discharge evoked by both volleys together was larger than either of the two individual responses, but less than their sum, i.e. occlusion was regularly observed. We have never observed that, on simultaneous combination of two antidromic volleys, one caused a diminution of the discharge produced by the other, i.e. we have never observed the inhibitory action described by Renshaw. However, a preceding antidromic volley has always diminished the discharge evoked by a subsequent volley over intervals as long as 100 msec (cf. Renshaw, 1946, Fig. 5), whether the two volleys were in the same or in different nerves.

It is a fairly general finding that, following a period of intense repetitive bombardment, synapses exhibit a prolonged increase in effectiveness, which has been called post-tetanic potentiation (Lloyd, 1949, 1952; Hagbarth & Naess, 1951; Eccles & Rall, 1951; Ström, 1951). Fig. 8 shows that there is also post-tetanic potentiation for the antidromic activation of Renshaw cells. Following an antidromic tetanus at 660/sec for 10 sec there is seen to be an increase both in the frequency and duration of the Renshaw cell discharge evoked by another antidromic volley in the same motor axons (Fig. 8, inset). The time course of the effect is plotted in Fig. 8, where the test volley was applied initially at about 2·5 sec intervals and later at about 4 sec intervals, and the total number of discharges is plotted for each test interval. The time course of the rise and decay of the post-tetanic potentiation resembles that observed for monosynaptic reflexes after a comparable conditioning tetanus (Lloyd, 1949; Eccles & Rall, 1951). One experimental series departed from the usual type illustrated in Fig. 8, the potentiation being maximum as early as 3 sec after the end of the tetanus, after which the potentiation declined much more rapidly than in Fig. 8 and after 30 sec was succeeded by a prolonged depression.

Location and orientation of Renshaw cells. Usually the first few spike potentials generated by discharges of Renshaw cells were superimposed on irregular

waves of negativity having approximately the same frequency (cf. Figs. 5A, 10A, lower record). In fact the proximity of the microelectrode to Renshaw cells was signalled by the prominence of this characteristic rhythmic waveform that followed immediately after the negative spike generated by antidromic invasion of the motoneurone. This decrementing rhythmic wave at about 1000/sec (cf. Fig. 10A, lower record) was first reported by Barakan, Downman & Eccles (1949) as an almost invariable sequel to the antidromic spike potential, and Lloyd (1951*a*) has also illustrated 'wavelets' following the antidromic spike potential.

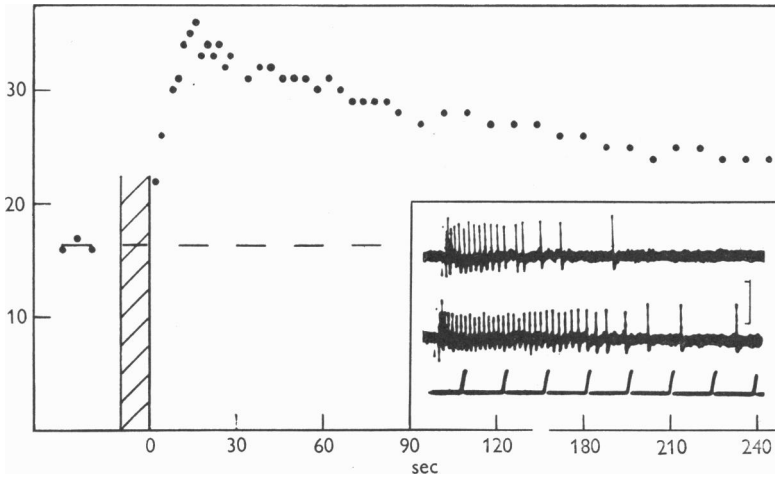

Fig. 8. The inset figure gives specimen records of Renshaw cell discharges evoked by an antidromic volley in the seventh lumbar ventral root before and during the post-tetanic potentiation that follows conditioning by an antidromic tetanus at 660/sec for 10 sec. Time is in 10 msec. Potential scale gives 0·5 mV. [In the main figure the time course of the post-tetanic potentiation is plotted. Each point gives the number of Renshaw cell discharges evoked by the antidromic volley at the indicated interval after the cessation of the conditioning tetanus. Initial control values are plotted at the extreme left, the mean level being given by the broken line. The tetanic period is shown by the hatched area.

As the microelectrode was slowly inserted through a region of the ventral horn rich in Renshaw cells, its passage in close proximity to several cells was signalled as one cell after another was seen to generate a characteristic sharp spike potential which rose above the background rhythmic wave as illustrated in Fig. 10A. Repeated observations of this nature provide convincing evidence that the background rhythmic wave is generated by the cumulative effects of discharges in a population of Renshaw cells, none of which lies sufficiently close to the microelectrode to contribute an independently recognizable spike. As indicated in Figs. 5A, 6E, 11F and 13B, Renshaw cells that were intensely excited had almost identical values for the latent period of the first discharge

and for the intervals between the first few discharges; hence the approximate superposition of these first discharges to give the composite wave-form. The progressive decrease in amplitude of the successive waves to eventual extinction (cf. Fig. 10A) would be expected to occur as the successive discharges of the various Renshaw cells gradually became more and more out of phase.

When this Renshaw cell wave-form was systematically recorded at 0·2 mm intervals along a series of parallel microelectrode tracks lying 0·2 mm apart in the transverse plane, it was possible to construct a map (Fig. 9B) showing the focus for maximum response, exactly as has been done for other potential waves (Eccles, Fatt, Landgren & Winsbury, 1954). But, in addition, such systematic investigations revealed a remarkable feature, a reversal of the phase of the rhythmic wave. As illustrated by records along one such microelectrode track (Fig. 9A), in the lateral and dorso-lateral regions of the spinal cord the rhythmic wave is of the same frequency but of reversed polarity to the rhythmic wave that is recorded in close proximity to the Renshaw cell focus (cf. Fig. 10A) and generally in the ventro-medial regions. The dotted line in the map illustrated in Fig. 9B indicates the boundary between these two inverse phases, i.e. it is the line of phase-reversal. The seventh record of Fig. 9A was recorded at such a region of phase-reversal, while the ninth record was close to the maximum for Renshaw cell activity. Lines of phase-reversal for the rhythmic waves have been previously plotted and closely resemble the line of Fig. 9B (cf. Barakan *et al.* 1949; Figs. 1 and 10, the lines formed by large dots). The rhythmic wave of inverted phase is particularly large on the dorso-lateral surface of the spinal cord, an observation which enables investigations to be conducted on Renshaw cell discharges without the complications of microelectrode technique. For example, a platinum wire electrode led the large potentials of Figs. 10A (upper record) and 15A, C from the dorso-lateral surface of the spinal cord.

The phase-reversal of the rhythmic wave from the ventro-medial to the dorso-lateral aspect of the spinal cord gives the general orientation of the extracellular currents that occur during the discharges of impulses by Renshaw cells. It has already been seen that the discrete negative spikes generated by discharges from individual Renshaw cells are synchronous with the negative phases of the waves recorded ventro-medially, and hence with the positive phases of the waves recorded dorso-laterally. Thus, when Renshaw cells generate impulses, current flows into them from sources situated dorso-laterally. Presumably this current in the external circuit is flowing from the axons into the bodies of the Renshaw cells, i.e. the axons run dorso-laterally from the bodies.

The great majority of the individually recognizable Renshaw cells have been situated close to the focus in the ventro-medial region of the ventral horn in close proximity to the paths of the emerging motor axons (cf. Balthasar, 1952).

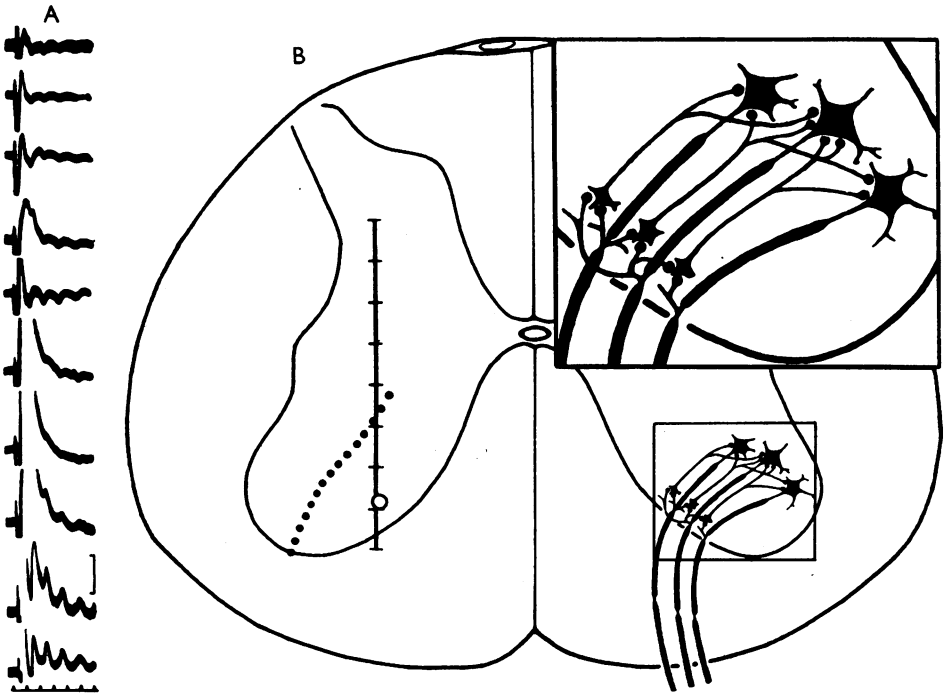

Fig. 9A. Potentials recorded from the seventh lumbar segment of the spinal cord in response to a maximum antidromic volley in the seventh lumbar ventral root. Each record is formed by the superposition of about 40 faint traces. The uppermost record is by a surface lead from the dorso-lateral surface. The remaining records are obtained at the depths indicated by short transverse lines along the track shown by the continuous line running dorso-ventrally in Fig. 9B. Time is in msec. Potential scale gives 0·5 mV for all but the uppermost record, where it gives 0·05 mV. Upward deflexions signal negativity of recording electrode relative to the indifferent lead.

Fig. 9B. Drawing of a transverse section of the spinal cord. In the left half the open circle indicates the maximum focus of Renshaw cell activity as detected by the systematic exploration described in the text. Also shown is the electrode track along which records of Fig. 9A were recorded. The dotted line separates the ventro-medial zone of Renshaw cell negativity from the dorso-lateral zone of Renshaw cell positivity (see text). On the right half is shown in schematic form the proposed nervous pathways (see details in inset) consisting of motor-axon collaterals, Renshaw cells and motoneurones. The Renshaw cells are located in the region from which most recordings from individual cells are obtained and their axons course therefrom in the direction that accounts for the phase-reversal of the rhythmic wave generated by their discharges (see text).

However, some have been observed more dorsally, even at the extreme dorsal limits of the motoneuronal area.

When Renshaw cells gave large spike potentials, it has been possible to keep the same potentials under observation even when the microelectrode was moved over a considerable distance. For example, in the series of Fig. 10B the microelectrode was moved inwards 25μ between each successive record. Actually the successive movements were only 12·5μ, but it is sufficient for illustration to show only alternate records. The largest spike response is seen

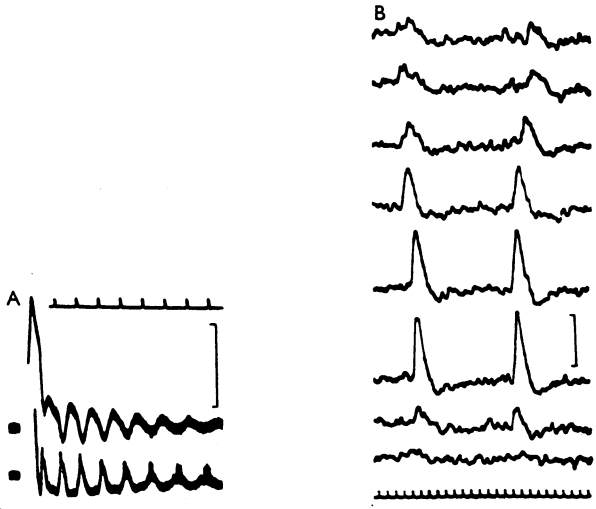

Fig. 10A. Simultaneous records (superposition of about forty faint traces) of potential waves evoked by an antidromic volley in the seventh lumbar ventral root and recorded from the dorsolateral surface of the cord (upper record) and in close proximity to a Renshaw cell (lower record). Upward deflexions signal negativity of the recording electrodes relative to the indifferent lead. Note that on the lower record a small Renshaw cell spike is superimposed on each rhythmic wave, but there is a progressive increase in the variability of timing of this spike. Time is in msec. Same potential scale gives 1 mV for lower record and 0·1 mV for upper record.

Fig. 10B. Series of records from a single Renshaw cell recorded by a microelectrode during a series of successive inward moves of 25μ. Each record shows two spike responses at approximately the same position. Time scale in 0·1 msec. Potential scale gives 0·5 mV.

in the sixth record as a diphasic wave with an initial negative phase of about 0·2 msec followed by a small but longer positive phase. Despite the background noise, it can be seen in all but the deepest record that there was no significant change in this time course over the whole range of depths, i.e. there was an initial sink even 125μ more superficial than the point in closest proximity to the Renshaw cell. With rare exceptions the time courses of the Renshaw cell spikes were similar to those of Fig. 10B (cf. Renshaw, 1946). However, this extracellular recording in a volume conductor gives merely the time courses of the external currents that flow during the discharge of impulses. For example, Fig. 10B

shows that there was a current into the body and dendrites of the Renshaw cell for about 0·2 msec, and then there was a small reverse current for about 0·6 msec. On the other hand, the intracellular recording of Fig. 6F–H gives a reliable measure of the time course of the spike potential, which is seen to have a total duration of about 0·7 msec, a value rather shorter than that for the motoneurone (Brock *et al.* 1952) Presumably in Fig. 10B the initial inward current ceased when the discharged impulse invaded the whole cell including the axon, and the subsequent outward current is attributable to the more delayed re-polarization of the axon. The large Renshaw cell spikes of Fig. 6A–E have the unusual feature of an initial positivity, which is possibly attributable to a small injury due to the very close proximity of the microelectrode. Exceptionally, also, an initial positive phase has been recorded about 100μ dorso-laterally to the zone of largest potentials. Presumably the microelectrode was then close to the axon of the Renshaw cell and so recorded the initial outward currents present at that site.

It is probable that the corresponding positive spike potentials around the ramifying axons of Renshaw cells were usually so small that they were lost in the background noise. However, as we have seen, summation of such potentials for the whole population of Renshaw cells may be assumed to give the rhythmic wave of inverted phase in the dorso-lateral region of the spinal cord. The much smaller voltage of this inverted wave-form (compare fifth and ninth records of Fig. 9A and the ten-fold difference between the voltages of two records of Fig. 10A) is in accord with this explanation.

C. *Pharmacological studies on Renshaw cells*

Investigations on chemical transmission at peripheral neuro-effector junctions have led to the postulate that the same chemical transmitting substance is employed at all junctions operated by a particular cell (Dale, 1934, 1952; Feldberg, 1950, 1952). This postulate has been employed in an attempt to discover the central synaptic transmitter for those dorsal root fibres that by an axon-reflex mechanism are responsible for peripheral vasodilatation (Hellauer & Umrath, 1948; Holton & Holton, 1952), but no satisfactory identification has yet been achieved. This same postulate leads to the expectation that the synaptic transmitter by which motor-axon collaterals activate Renshaw cells is identical with that by which, at their peripheral terminals, the same motor-nerve fibres activate muscle fibres, i.e. even as the peripheral transmitter is acetylcholine, so is the central transmitter. This expectation has been tested pharmacologically by three classes of drugs: those that depress cholinergic transmission; those that prolong it by acting as anticholinesterases; and the suspected transmitter, acetylcholine. The present account will be restricted to those pharmacological observations that are of significance in establishing the cholinergic activation of Renshaw cells by motor-axon

collaterals. Further pharmacological investigation will be described in a later paper (from this laboratory).

Depressants of cholinergic transmission. By far the most intense depression has been produced by dihydro-β-erythroidine. Intravenous injection of a very small dose (0·1 mg dihydro-β-erythroidine hydrobromide/kg) has invariably

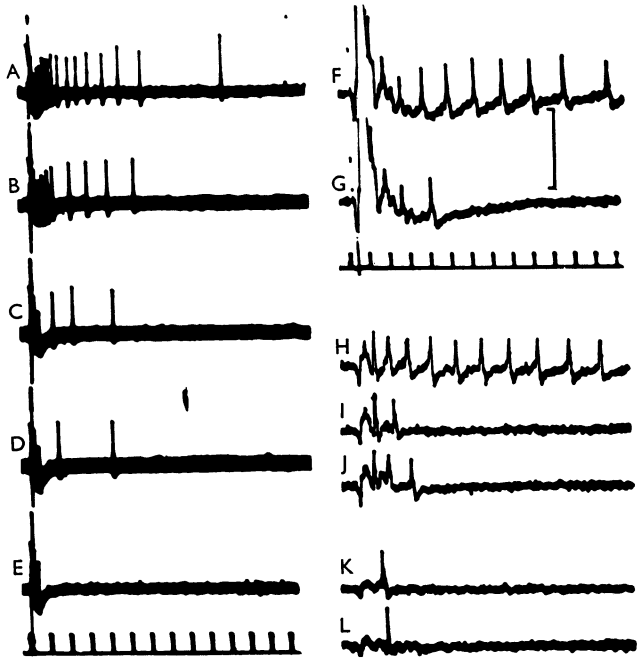

Fig. 11. A shows a Renshaw cell response to a maximum antidromic volley in the seventh lumbar ventral root. B–E show the development, as described in the text, of depression following the intravenous injection of 0·1 mg dihydro-β-erythroidine hydrobromide/kg body weight. Time scale is in 10 msec. F and G are the same series, but at a faster sweep speed, and correspond to A and E respectively. Note the first Renshaw cell spike on the declining phase of the soma spike potential. Time scale is in msec. H–L show the depression of the response of another Renshaw cell produced by the intravenous injection of a ten-fold larger dose (1 mg/kg). Before the injection H and K are the responses of the same cell to maximum antidromic volleys in biceps-semitendinosus and in semimembranosus motor axons respectively, while I and L show the responses to these volleys obtained during maximum depression. J is recorded 1 hr later than I to show the small degree of recovery of the response. Time is the same as for G. Potential scale gives 1 mV for all records.

had a depressant action which has reached a maximum within a few seconds and has persisted for hours. For example, very little depression had occurred in Fig. 11B 10 sec after the injection, but Fig. 11C and D at about 15 and 20 sec after injection show the rapid onset and by 30 sec the maximum depression was attained (Fig. 11E). Actually, as shown in the faster record (Fig. 11G), the Renshaw cell still gave four early discharges. Careful comparison

with the initial control at the same sweep speed (Fig. 11F) reveals typically that this small dosage of dihydro-β-erythroidine had produced absolutely no change in the timing of the first three responses. As illustrated in Fig. 11H and I, even a ten-fold larger dosage regularly failed to suppress the first two discharges of an intensely activated Renshaw cell, and furthermore it also failed to suppress the single discharge produced by a much less effective anti-

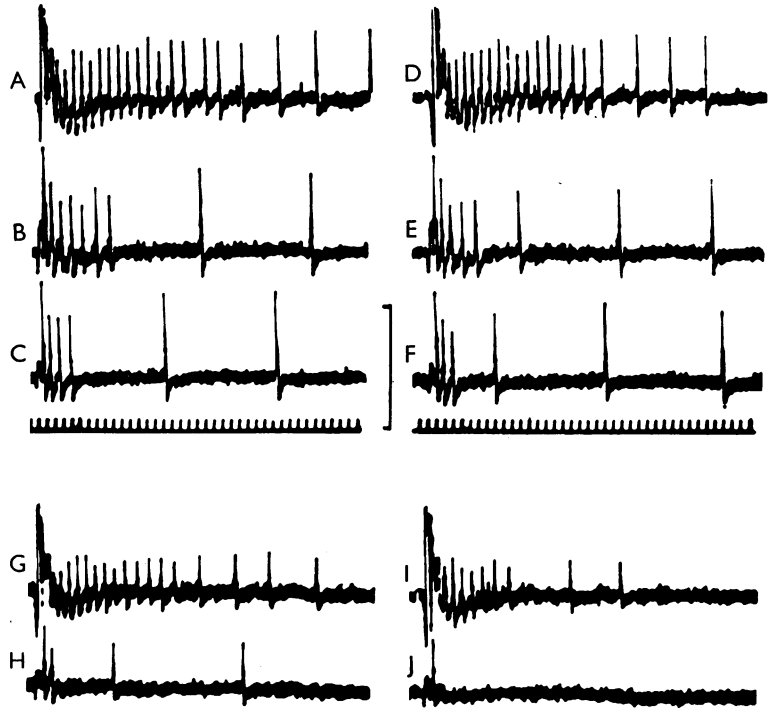

Fig. 12. A, B and C show spike responses of a Renshaw cell to maximum antidromic volleys in motor fibres of biceps-semitendinosus, gastrocnemius and flexor digitorum longus nerves respectively. D, E, F are the corresponding responses at 2 min after intravenous injection of 1 mg D-tubocurarine chloride/kg body weight. Similarly G–J show the action of the intravenous injection of 2 mg atropine sulphate/kg body weight. G and H are the responses to biceps-semitendinosus and gastrocnemius volleys before, and I and J after the injection. Time is in msec for all of the figure. Potential scale gives 1·0 mV.

dromic volley (Fig. 11K, L). Thus it has been an invariable finding that the low-frequency later discharges are very sensitive to depressant drugs, while the initial discharges are very insensitive. The very slow recovery from such a large dosage is seen in Fig. 11J, where the number of discharges had increased only from 2 to 3 at 1 hr after the injection.

The much less potent action of atropine even in very large doses (2 mg atropine sulphate/kg body weight) and the ineffectiveness of D-tubocurarine (1·0 mg D-tubocurarine chloride/kg body weight) are illustrated in Fig. 12.

Anticholinesterase drugs. When given by intravenous injection, eserine has greatly prolonged the repetitive discharges evoked by an antidromic volley, but has had no appreciable action on the initial period of high-frequency discharge. For example, the lengthening is shown in Fig. 13A and C, while the initial discharges were not significantly altered (Fig. 13B and D). The action of eserine has the slow onset characteristic of anticholinesterase effects. In Fig. 13C, 100 sec after injection of 0·2 mg eserine sulphate/kg, the discharge was less than 200 msec in duration, while 400 sec later (Fig. 13E) the discharge was almost 600 msec in duration. However, at 1000 sec after the injection the duration had shortened to about 450 msec. A further injection of 0·5 mg eserine sulphate/kg then caused the much longer and more intense discharge of Fig. 13F, which by slower recording was found to persist for at least 2 sec. Still

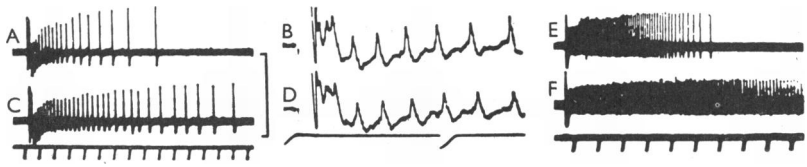

Fig. 13. A and B show Renshaw cell response to a maximum antidromic volley in the seventh lumbar ventral root at slow and fast sweep speeds, while C and D show the respective responses 100 sec after the intravenous injection of 0·2 mg eserine sulphate/kg body weight. Time scales are in 10 msec. E shows the full development of the prolonged discharge due to this injection, and F the further prolongation due to the injection of an additional 0·5 mg/kg. Time scale is in 100 msec. Potential scale gives 1·0 mV for all of the figure.

larger doses of eserine, e.g. 1·0 mg/kg, caused a spontaneous background discharge of Renshaw cells, so it was then impossible to assess the duration of the discharge evoked by a single antidromic volley. In contrast to prolonging the response of Renshaw cells to a single volley, eserine suppressed the response to successive volleys in a repetitive series (cf. section *D*).

Tetraethylpyrophosphate (TEPP) and the dimethylcarbamate of 3-hydroxy-2-dimethylaminomethylpyridine dihydrochloride (Roche, NU2126) were equally as effective as eserine. Prostigmine, however, was virtually ineffective even in high doses (1·0 mg prostigmine bromide/kg body weight).

Acetylcholine. Acetylcholine has been injected into the spinal cord by employing the intra-arterial technique (cf. Holmstedt & Skoglund, 1953) when the microelectrode had been inserted close to a Renshaw cell which was indicated by the rhythmic spike potentials evoked by an antidromic volley. As illustrated in Fig. 14A some Renshaw cells were readily excited by relatively small injections of acetylcholine. Identification was established by the similarity of the spike potentials, when observed in detail at fast sweep speeds (Fig. 14G, H), to those generated by an antidromic volley (Fig. 14F). The double discharges seen in Fig. 14A, B, G were an unusual feature. The threshold

for the cell illustrated in Fig. 14A was 8μg acetylcholine chloride. Larger injections of acetylcholine gave higher frequencies and longer durations of the discharge. Frequencies as high as 100/sec have been evoked by relatively large injections (100μg), and the duration of the discharge was then as long as 10 sec. The upper row of Table 2 gives the total number of impulses discharged by a Renshaw cell in response to graded dosage with acetylcholine.

Approximately half the Renshaw cells that we have tested have been caused to discharge by injections of not more than 20μg acetylcholine chloride. Some of the remainder have failed to respond even to 200μg. Larger doses could not be given because contractions of the lumbar musculature produced troublesome artifacts and movements. Hence we have the surprising observation that, as judged by the action of injected acetylcholine, there was a great variability between different Renshaw cells, and yet all behaved identically when their responses to antidromic volleys were subjected to the actions both of drugs which block cholinergic transmission and of anticholinesterases which prolong cholinergic action.

As would be expected, the effectiveness of a given injection of acetylcholine was always greatly increased by prior intravenous injection of eserine. For example, comparison of Fig. 14B, C with Fig. 14A shows the increased frequency of response evoked by injection of 30μg acetylcholine chloride after injection of 0·2 mg eserine sulphate/kg. A further injection of 0·5 mg eserine sulphate/kg increased the frequency still further to almost 100/sec (Fig. 14D). The second row of Table 2 shows the great increase in the total number of impulses generated by a given dosage of acetylcholine, and it also shows the lowering of threshold, 2μg being as effective as 8μg before the eserine injection.

Fig. 14E and Table 2 further illustrate the depressant action which a small dose of dihydro-β-erythroidine hydrobromide (0·1 mg/kg) exerts on the response to injected acetylcholine. The frequency of discharge suffered a five-fold diminution, and was greatly curtailed in duration, resembling a just-threshold response. Comparison of the third with the second rows of Table 2 shows that there has been approximately a ten-fold diminution in sensitivity to injected acetylcholine, e.g. 30μg then gives a response of about the same order as 3μg beforehand.

D. *Pharmacological investigations on the inhibitory pathway*

The pharmacological investigations of the preceding section were specifically designed to test the postulated cholinergic transmission at the synapses on Renshaw cells. Related investigations aid in establishing other features of the inhibitory pathway.

It has already been seen that the rhythmic wave recorded from the dorso-lateral regions of the spinal cord is of the same frequency but of inverted

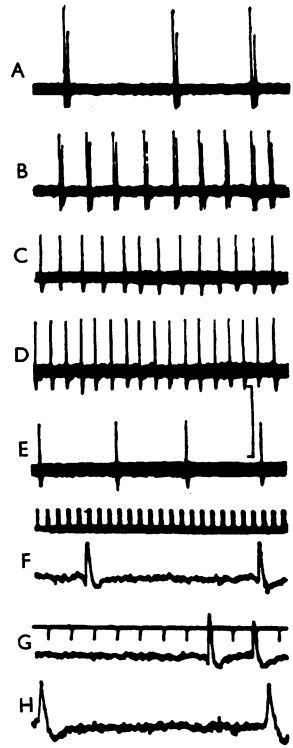

Fig. 14. A–E and G, H are records of Renshaw cell discharges evoked by intra-arterial injections of 30μg acetylcholine chloride as described in the text. Each record is a brief excerpt from the most intense part of a discharge that persisted for several seconds. B and C show responses at 10 and 18 min after the intravenous injection of 0·2 mg eserine sulphate/kg, while D is the response 10 min after a further injection of 0·5 mg eserine sulphate/kg. Between D and E 0·1 mg dihydro-β-erythroidine hydrobromide/kg body weight was injected intravenously. Time scale is in 10 msec. F–H are much faster records, showing in F the later part of a Renshaw cell response evoked by an antidromic volley. In G one of the double discharges is evoked as in A, and in H two of the rhythmic discharges are evoked as in D. Time scale is in msec. Potential scale gives 0·5 mV.

TABLE 2. Numbers of impulses evoked in a quiescent Renshaw cell by intra-arterial injection of acetylcholine

	Amounts of acetylcholine chloride injected in μg								
	2	3	5	8	10	20	30	60	90
Initial response	—	—	—	12	43	118 100	126	233	—
After injection 0·7 mg eserine sulphate/kg	12 14	31	75	—	172 229	573	790	—	—
After further injection 0·1 mg dihydro-β-erythroidine hydrobromide/kg	—	—	—	—	—	—	20	173	249

polarity to the larger rhythmic wave in the ventro-medial regions (cf. Fig. 9A), which is itself synchronized with the initial discharges of the Renshaw cells (cf. Fig. 10A). It was postulated that the rhythmic discharges of the whole population of Renshaw cells produced a current from the axons to the cell bodies lying ventro-medially, the discharges initially being sufficiently synchronized to give the rhythmic wave whose decrementing amplitude would be largely attributable to increasing asynchronism of the individual components. This postulate receives further support from pharmacological investigations. Comparison of Fig. 15C with A shows that intravenous injection

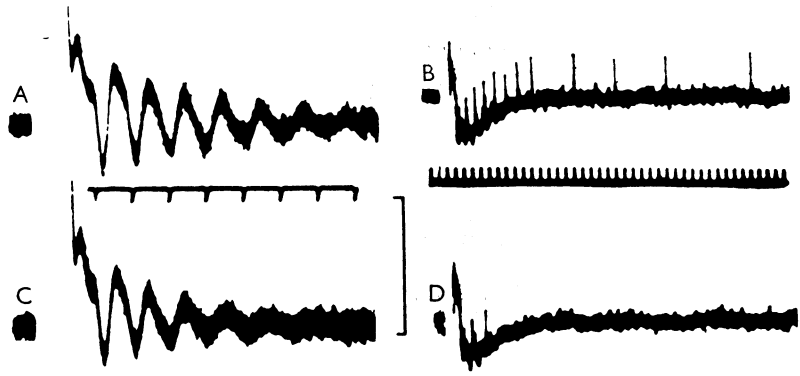

Fig. 15. A and B give the potentials evoked by a single antidromic volley (in the seventh lumbar ventral root) and recorded respectively on the dorso-lateral surface of the spinal cord and in proximity to a Renshaw cell in the ventral horn. C and D give corresponding potential records after the injection of 0·2 mg dihydro-β-erythroidine hydrobromide/kg body weight. Both time scales are in msec. The same potential scale gives 0·1 mV for A and C and 1·0 mV for B and D. A and C are formed by the superposition of about 40 faint traces.

of 0·2 mg of dihydro-β-erythroidine hydrobromide/kg depressed the later stages of the rhythmic wave, while correspondingly the much slower records of a Renshaw cell discharge showed the usual suppression of the later discharges (Fig. 15B, D). The relatively small diminution of the earlier stages of the rhythmic wave corresponds with the resistance of the initial Renshaw cell discharges to the depressant action (cf. Fig. 11).

That a relation exists between the surface oscillatory wave and the repetitive discharge of Renshaw cells is confirmed by the modification produced in both by anticholinesterases during repetitive motor-axon stimulation. In the records illustrated in Figs. 5, 6, 11, 12, 13 there has been an interval of 3·5 sec between each successive antidromic volley, and with such an interval the responses of Renshaw cells to successive volleys have been well maintained even when under the influence of heavy anticholinesterase dosage. In Fig. 16 much faster repetition rates have been employed, 2, 8 and 27/sec respectively

for the records in the three columns. Before dosage with the anticholinesterase TEPP (Fig. 16A to E), the first three discharges of the Renshaw cell correspond as usual with the first three downward deflexions of the surface record, which is shown below the simultaneously recorded Renshaw cell response. Responses at 2 and 8/sec are virtually identical (cf. Fig. 16A and C, B and D), but at 27/sec (Fig. 16E) the first discharge has a longer latent period and the frequency is lower. Moreover, the rhythmic wave is not so well maintained with the surface record in Fig. 16F, as compared with Fig. 16B and D. Comparison of Fig. 16G, H with A, B reveals that, even with repetitive

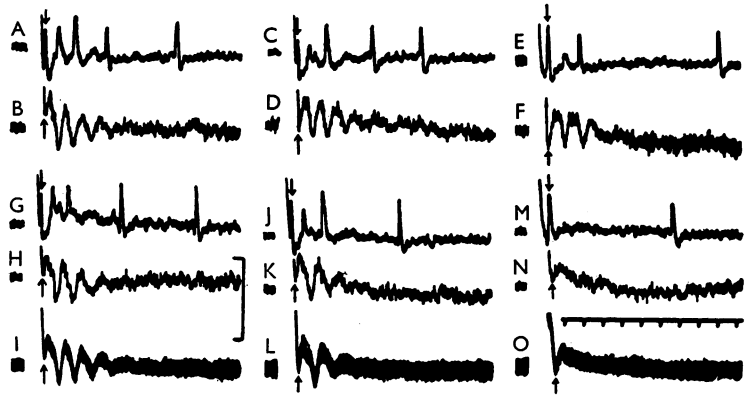

Fig. 16. A and B give simultaneously recorded potentials evoked by a single antidromic volley (in seventh lumbar ventral root) during a repetitive series at 2/sec, A showing a Renshaw cell response and B potential waves led from the dorso-lateral surface of the spinal cord. C, D and E, F are similar, but the repetitive series are at 8/sec and 27/sec respectively. G and H are similar to A and B respectively, but after the injection of 0·2 mg TEPP/kg. I is similar to H, but is formed by 40 superimposed faint traces in order to smooth out noise. J, K and L likewise correspond to C and D; and M, N and O to E and F. The first spikes discharged by the Renshaw cell and the corresponding initial positive waves in the surface records are marked by arrows in all records. The second spikes are usually much smaller. In all records a gap occurs during the large initial motoneuronal spike potentials. Further description is in text. Time scale is in msec. Potential scale gives 1·0 mV for Renshaw cell records and 0·1 mV for surface records.

stimulation as fast as 2/sec, there was after TEPP dosage very little depression in the Renshaw cell response, as shown both by direct recording from one and by recording the inverted surface wave that was generated by the whole population of Renshaw cells. This surface response is better seen in the superimposed traces of Fig. 16I, L and O. However, comparisons of Fig. 16J with C and K or L with D reveal that at the repetition rate of 8/sec the Renshaw cell response was considerably depressed by the TEPP, being comparable with the normal responses at 27/sec (Fig. 16E, F). A much greater depression after the TEPP dosage occurred with the repetition rate of 27/sec (Fig. 16M, N, O),

there being only one initial discharge both in the Renshaw cell response and in the surface response. Intermediate levels of response were observed with repetition rates of 4 and 12/sec.

It may be concluded that the surface oscillatory wave is produced by repetitive Renshaw cell activity. It should also be pointed out that the depressant effect of an anticholinesterase is a well-known phenomenon with repetitive activation of the neuro-muscular junction (Bacq & Brown, 1937; Cowan, 1938), where it is largely attributable to the accumulation of acetylcholine at the junctional region; hence the observations illustrated in Fig. 16 further support the postulate that Renshaw cells are activated by cholinergic synapses.

It remains to remove the possibility that the surface wave might be due to the repetitive wave component of the hyperpolarization produced in motoneurones (cf. Figs. 2G, 3H, 4A). Strychnine adequately differentiates the two phenomena. As noted earlier strychnine reduces the hyperpolarization in the motoneurone (Fig. 4D–H). At the same time the Renshaw cell discharge was slightly increased and the surface wave showed no significant change.

If the impulses discharged by Renshaw cells are responsible for generating the antidromic IPSP of motoneurones, it would be expected that modification of the Renshaw cell discharge by pharmacological agents would be associated with corresponding alterations in the time course of the antidromic IPSP. The predicted effects are illustrated in Fig. 17. Intravenous injection of 0·1 mg dihydro-β-erythroidine hydrobromide/kg caused little depression in the initial rate of development of the IPSP, but the summit was earlier and lower and the recovery therefrom much more rapid (cf. Fig. 17A with C and B with D). This is precisely the effect that would be expected on account of its depressant action on the later rather than the earlier Renshaw cell discharges (cf. Fig. 11). Since dihydro-β-erythroidine has been observed to have no action on direct IPSP's, it may be presumed that the whole effect seen in Fig. 17C and D is attributable to its depressant action on Renshaw cell discharges. As would be expected from the effect on Renshaw cell discharge (Fig. 13), the anticholinesterase, eserine, had the opposite action on the antidromic IPSP. The rising phase to the summit was not appreciably altered, but thereafter the time course was greatly slowed (Fig. 17E, F), as may be seen in the slower recording of Fig. 17G, where the time from the summit of the IPSP to half decay was a three-fold lengthening from the corresponding value for Fig. 17E. Such a change may be satisfactorily attributed to the large increase which eserine brings about in the later stages of a Renshaw cell discharge, the initial discharges being virtually unaltered (Fig. 13).

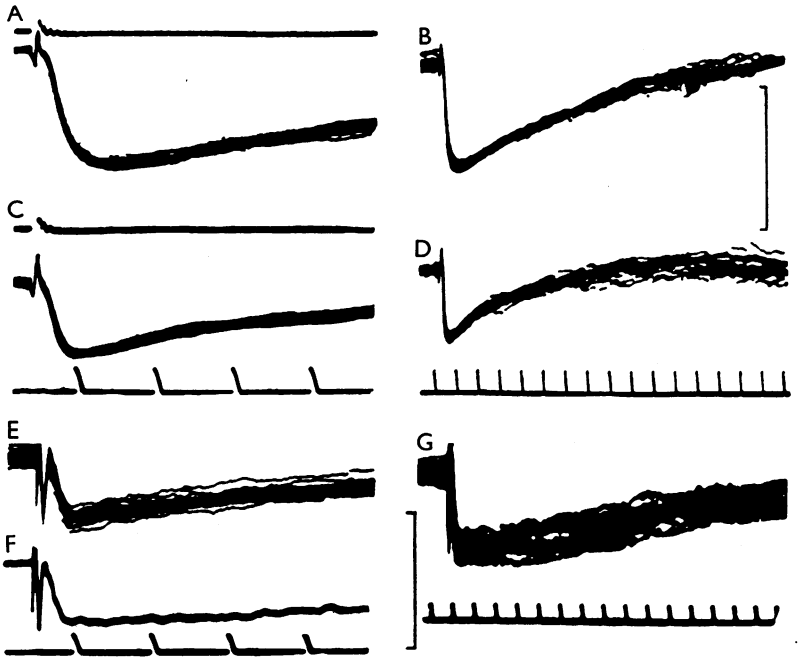

Fig. 17. Intracellularly recorded inhibitory post-synaptic potentials evoked in a motoneurone by a single antidromic volley in the seventh lumbar ventral root. A, B are before and C, D after the injection of 0·1 mg dihydro-β-erythroidine hydrobromide/kg body weight. Time scales in 10 msec are shown below the corresponding records. Potential scale gives 10 mV. E and F show IPSP's similarly evoked in another experiment, E before and F 5 min after the injection of 0·3 mg eserine sulphate/kg body weight. G is the same as F but at a slower sweep speed. Time scales are in 10 msec. Potential scale gives 5 mV. All records except F are formed by the superposition of about 40 faint traces.

DISCUSSION

The anatomical pathway

In our usual experiments Renshaw cells have been excited to discharge by stimuli applied either to ventral roots or to motor nerves, the appropriate precautions being taken to prevent impulses in afferent fibres from entering the spinal cord. By graduating the strength of stimulation it has been shown that activation of Renshaw cells is caused solely by impulses in nerve fibres whose range of thresholds corresponds to the large motor-nerve fibres (cf., for example, Fig. 6B–E). However, nerve volleys entering the spinal cord via the dorsal roots frequently evoke discharges of Renshaw cells (Renshaw, 1946). Discharges occurring after a short latent period have been observed to be associated with large reflex discharges from motoneurones, and the depression of this reflex by deeper anaesthesia has abolished the Renshaw cell response. Such Renshaw cell activation may therefore be assumed to be secondary to

the reflex discharge of impulses outwards along the motor-nerve fibres, the impulses having presumably the same action as impulses propagating antidromically up these fibres. However, volleys in the small group III afferent fibres often evoke discharges of Renshaw cells which have a much longer latent period and which are not secondary to the discharge of impulses from motoneurones. While this aspect of Renshaw cell activity has not been fully explored, it does indicate that motor-axon collaterals do not provide the only excitatory pathway to Renshaw cells. In addition, Renshaw (1946) reported a slight inhibitory action of antidromic volleys on Renshaw cells, but this report requires confirmation.

The negative results of the facilitation experiments illustrated in Fig. 7 have been taken to exclude the possibility that Renshaw cells are activated by the antidromic volley after it has traversed the cell body and invaded the dendrites of the motoneurones. On neuro-histological grounds the only alternative would appear to be activation by means of the axon collaterals. However, this postulate is made without any histological evidence that these collaterals end synaptically on special interneurones in the ventral horn. The usual electrophysiological location of Renshaw cells in the ventro-medial zone of the ventral horn may be taken as additional evidence supporting their synaptic stimulation by means of motor-axon collaterals; for it is in this zone that these collaterals arise from the motor axons and terminate by extensive ramification (Cajal, 1909, Fig. 133).

Though interneurones are relatively numerous in the ventral horn, outnumbering motoneurones by three to one (Balthasar, 1952), it has been possible to find only one account in the literature which may relate specifically to Renshaw cells. After section of the ventral roots, Sprague (1951, Figs. 5, 10) found that the chromatolytic reaction occurred not only in large cells which undoubtedly are motoneurones but also to a variable degree in many small cells which, in the lower lumbar region, were concentrated in the extreme ventro-medial zone of the ventral horn. This is precisely the focal region for Renshaw cells (cf. Fig. 9B). He suggested that these cells (designated Type II cells) were small motoneurones, i.e. the motoneurones of small motor fibres innervating muscle spindles. However by antidromic activation it has been shown that the small motoneurones of a muscle are located in proximity to the large motoneurones and not in the ventro-medial zone. It therefore seems probable that the Type II cells are Renshaw cells. Sprague himself states that the atypical chromatolytic changes do not provide conclusive evidence that the Type II cells are motoneurones.

The present experiments confirm Renshaw's (1946) conclusion that impulses in many motor fibres activate any one Renshaw cell. This has been shown both by the effects of varying the size of the antidromic volley in any one motor nerve and by the excitatory action of volleys in many different motor nerves

(Fig. 6). Since the latent period of activation (0·6–0·7 msec) is about the minimum duration for a single synaptic relay in the central nervous system, there can be no interpolated neurones. As judged by the steady decline in the rate of discharge of a Renshaw cell, the activating transmitter substance reaches a maximum within 1 msec of the invasion of the terminals of the motor-axon collaterals, and thereafter it steadily declines along a time course which is indicated in Fig. 6I. There is no need to postulate that the prolonged discharge is due to delay-paths from motor-axon collaterals through chains of interneurones to Renshaw cells.

It has already been pointed out that the antidromic IPSP has precisely the latency and time course that would be predicted if it were generated by the discharges of Renshaw cells. It has further been observed that impulses in many motor-nerve fibres sum in producing the IPSP of a motoneurone. Undoubtedly this convergence on to a motoneurone is largely attributable to the convergence on to individual Renshaw cells. However it is also necessary to postulate that several Renshaw cells contribute to the IPSP of a motoneurone. The rhythmic discharges of Renshaw cells undoubtedly give the rhythmic wave-form seen on the rising phase and summit of the IPSP (Figs. 2G, 3H, 4A, B). If this rhythmic wave-form were due to the rhythmic discharges of a single Renshaw cell, it should become accentuated as the frequency declines, but the reverse occurs. It is therefore necessary to assume that the rhythm is smoothed out because it is generated by the discharges of many Renshaw cells which progressively become out of phase, exactly as occurs with the rhythmic waves recorded on the surface and in the spinal cord (cf. Figs. 9A, 10A).

The antidromic IPSP may begin after a central delay as brief as 1·1 msec, which is only 0·5 msec after the initiation of the earliest spike in a Renshaw cell discharge. However by recording the negative spike of a Renshaw cell extracellularly (Fig. 10B), it is seen that about 0·2 msec is required for the invasion of the whole cell. Hence 0·3 msec remains between the invasion of the Renshaw cell terminals and the commencement of the IPSP, which agrees closely with the value determined for the direct inhibitory pathway (Eccles, Fatt & Landgren, 1954). Even when the central delay for the antidromic IPSP is as long as 1·8 msec, there is no time for the interpolation of a neurone between Renshaw cells and motoneurones, for with weak activation of Renshaw cells the latent period for their discharge may be longer than 1 msec (Fig. 5C, D, E; Fig. 6B, C). The prolonged time course of the antidromic IPSP is sufficiently explained by the prolonged repetitive Renshaw cell discharge.

Thus the anatomical pathway may be drawn as on the right side of the transverse section shown in Fig. 9B. Motor-axon collaterals converge on to Renshaw cells and have an excitatory action thereon. Renshaw cells in turn converge on to motoneurones on which they have an inhibitory action.

The synaptic events

The fact that motor-nerve fibres produce and liberate acetylcholine at their peripheral contacts with muscle fibres makes it probable that acetylcholine is also the transmitter substance at the synapses which motor-axon collaterals make with Renshaw cells (Dale, 1934, 1952; Feldberg, 1950, 1952). The pharmacological investigations illustrated in Figs. 11–17 give strong confirmation to this postulate. Furthermore the investigations illustrated by Fig. 17 demonstrate that antidromic volleys in motor axons generate IPSP's in motonerones exclusively through the mediation of Renshaw cells, for the changes produced in the antidromic IPSP by dihydro-β-erythroidine and by eserine correspond precisely to the changes which these drugs produce in the discharge of Renshaw cells (Figs. 11, 13, 14, 15).

Various experimental procedures have indicated that the inhibitory synapses which Renshaw cells make with motoneurones resemble the synapses made by other inhibitory pathways to motoneurones. All the synapses are similarly affected by strychnine. Furthermore the potentials are similarly affected by the injection of ions into the cell and by the passage of currents across the surface membrane (cf. Coombs *et al.* 1953).

Conclusions

The conclusions derived from these experimental observations are illustrated in Fig. 18 which gives the successive physiological events and relates them to a diagrammatic representation of the anatomical pathway. The single impulse in the motor-axon collateral (Fig. 18A) causes the liberation of acetylcholine at synapses on Renshaw cells. The persistence of this acetylcholine (Fig. 18B) effects a prolonged depolarizing action on the Renshaw cell (cf. Fig. 6I) with the consequent generation of a repetitive discharge of impulses (Fig. 18C; cf. Fig. 6G) that gradually slows from a very high initial frequency. The latent period for the first discharge (0·6 to 0·7 msec) is in good agreement with the shortest values obtaining for other types of monosynaptic transmission. At the inhibitory synapse on the motoneurone each impulse from a Renshaw cell is shown in Fig. 18D liberating, after a delay of about 0·4 msec, the inhibitory transmitter substance, which has a characteristic brief duration of about 2 msec (Eccles, Fatt & Landgren, unpublished observations), and which in turn generates after a very brief delay the IPSP that has much the same time course as the IPSP observed with direct inhibition (Brock *et al.* 1952; Coombs *et al.* 1953). At the high initial frequencies there is fusion of the successive responses to give a considerable summation of the IPSP's, but for any one synapse this fusion will decrease as the frequency declines (Fig. 18E). However, as already suggested, the smooth contour of the IPSP beyond its summit (Figs. 2G, 3H, I) indicates that the discharges of several

Renshaw cells are responsible for generating the IPSP on any motoneurone, the asynchronism of the various discharges effecting a smoothing of the wave-form as indicated in Fig. 18F. The sites of action of the blocking agents dihydro-β-erythroidine and strychnine are also indicated in Fig. 18 between B and C and between D and E respectively, i.e. it is postulated that blockage occurs by interference with the action of the respective synaptic transmitters.

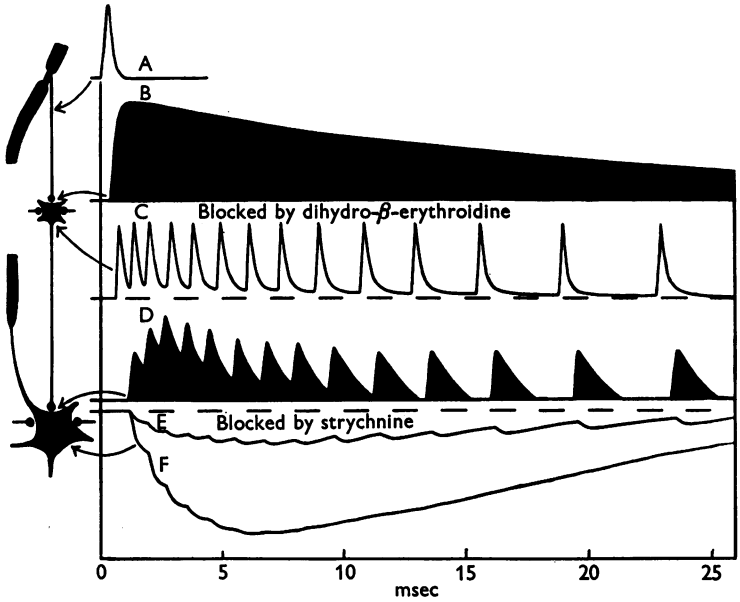

Fig. 18. Diagram summarizing the postulated sequence of events from an impulse in a motor axon to the inhibition of a motoneurone. All events are plotted on the time scale shown below and the corresponding histological structures (cf. Fig. 9B) are shown diagrammatically to the left (note indicator arrows). The six plotted time courses are for the following events: A, the electrical response of impulse in motor-axon collateral; B, the effective concentration of the acetylcholine which it liberates at a synaptic terminal; C, the electrical response evoked in a Renshaw cell by the cumulative effect of acetylcholine at many synapses, showing impulses superimposed on a background depolarization (cf. Fig. 6G); D, the effective concentration of inhibitory transmitter substance which these impulses liberate at a synaptic terminal of the Renshaw cell, showing summation at the high initial frequency; E, the IPSP generated in the motoneurone by the Renshaw cell discharge and the inhibitory transmitter shown in C and D, respectively; F, the aggregate IPSP evoked in a motoneurone that is repetitively bombarded by many Renshaw cells which progressively become more asynchronous so smoothing the latter part of the ripple shown in E. The morphological diagram to the left shows converging synapses both on the Renshaw cell and on the motoneurone (cf. Fig. 9B).

The inhibitory action which an antidromic volley exerts on motoneurones is sufficiently explained by the synaptic inhibitory pathway through Renshaw cells. Certainly the electric field explanation of Brooks *et al.* (1950) and Lloyd (1951*b*) is in good agreement with the temporal course of the inhibition. But

its quantitative aspect has been in doubt, and the present experiments establish that at best it is of secondary significance. For example in Figs. 1N and 3B, D no positivity is detectable when the electrode is withdrawn to a just-extracellular position. No voltage scale is given for the electric field potentials recorded by Lloyd (1951*b*), but the background noise level indicates a magnitude measurable in hundredths of mV, which is the magnitude recorded by Brooks *et al.* (1950). Only a fraction of this voltage drop would appear across the motoneuronal membrane to produce a hyperpolarization and consequent inhibition by field action. On the other hand the inhibitory synaptic potentials generated by a maximum antidromic volley in a ventral root usually attain several millivolts, i.e. the synaptic mechanism would be as much as one hundred times as effective as the field effect. Thus the electric field theory can be discounted on quantitative grounds until experimental investigation has established that an effective inhibition is thereby produced in addition to that arising from the synaptic mechanism demonstrated in this paper. It is further suggested that a search for synaptic mechanisms should be made in other instances in which neuronal interactions in the central nervous system have been attributed to the action of electrical fields.

The functional significance of this inhibitory pathway from motor axons to motoneurones remains obscure. As judged by the size of the IPSP, the intensity of the inhibition is often relatively high, being with many motoneurones larger than the direct IPSP. But on the other hand no antidromic IPSP has been detectable with about one-fifth of the motoneurones that we have investigated. We would agree with Renshaw that no functional meaning can yet be given to this potent and widely distributed inhibitory action, except in so far as it would exercise a generalized suppressor function (cf. Holmgren & Merton, 1954), which would be of importance in the limitation of such widely distributed and intense motoneuronal activity as, for example, occurs in convulsions. The long duration of the synaptic excitatory action on Renshaw cells (cf. Fig. 18B) would fit them particularly well for summing the individual excitatory actions of impulses which are discharged at the relatively low frequencies characteristic of motoneurones.

In addition Renshaw cells may also lie in other inhibitory pathways to motoneurones. This has been shown to obtain for impulses arising in group III afferent fibres. Possibly, too, Renshaw cells may be activated by descending pathways that have an inhibitory action on motoneurones, e.g. from inhibitory areas of the bulbar reticular formation (Magoun & Rhines, 1946; Niemer & Magoun, 1947). It is further possible that Renshaw cells may make synaptic contact with other neurones as well as with motoneurones.

SUMMARY

1. Intracellular recording from motoneurones in the lumbar region of the cat's spinal cord has revealed that volleys of impulses in motor axons generate a hyperpolarization of the motoneuronal membrane which has all the features of an inhibitory post-synaptic potential. A particular motoneurone is affected by impulses in many different axons supplying different muscles, but its own axon has no special action in this regard. Both the distribution of the axons which effect a hyperpolarization of the motoneurones supplying a given muscle and the time course of the hyperpolarization show that a satisfactory explanation is hereby provided for the antidromic inhibitory effect discovered by Renshaw (1941).

2. In addition to generating this inhibitory post-synpatic potential, impulses in motor axons set up a prolonged repetitive discharge of special interneurones in the ventral horn, which was first described by Renshaw (1946). The activation of these interneurones (designated Renshaw cells) is not caused by impulses propagating over the somas and dendrites of motoneurones; it is therefore postulated that their activation takes place via the collaterals of motor axons.

3. In conformity with Dale's principle (1934, 1952) that the same chemical transmitter is released from all the synaptic terminals of a neurone, pharmacological investigation has indicated that acetylcholine mediates the excitation of Renshaw cells by impulses in the collaterals of motor axons just as it mediates the excitation of muscle fibres at the peripheral terminals of the same axons. For example the transmission to Renshaw cells is readily depressed by dihydro-β-erythroidine and to a lesser extent by atropine and is greatly prolonged by such anticholinesterases as eserine and TEPP. However, transmission is not affected by either D-tubocurarine or prostigmine. Further evidence relating to the transmission is that Renshaw cells are caused to fire repetitively by the administration of acetylcholine via the arterial blood supply of the spinal cord. There is however a wide range of variability of Renshaw cells in their response to acetylcholine thus applied. As would be expected the sensitivity of Renshaw cells to injected acetylcholine is greatly increased by eserine and depressed by dihydro-β-erythroidine.

4. Experimental investigation establishes that the antidromic inhibitory potential of motoneurones is produced through the mediation of Renshaw cells. The latent period and time course of the inhibitory potential are in precise accord with this explanation. Furthermore depression and shortening of the Renshaw cell discharge by dihydro-β-erythroidine and its prolongation by anticholinesterases are accompanied by corresponding changes in the inhibitory potential.

5. The proposed anatomical pathway is shown in Fig. 9B. Motor-axon collaterals converge on to and make synaptic contacts with Renshaw cells whose

axons in turn converge on to and make synaptic contacts with motoneurones at the same segmental level. Systematic mapping of electric potential fields in the spinal cord reveals that Renshaw cells are concentrated in the ventro-medial zone of the ventral horn and send their axons dorso-laterally towards the motoneurones. The repetitive spikes which an antidromic volley generates in Renshaw cells are initially so well synchronized that they fuse to give a rhythmic potential wave at about 1000/sec. When recorded dorso-laterally in the spinal cord this wave has a polarity which is the inverse of the polarity in the ventro-medial zone where it arises from the repetitive discharges of Renshaw cells.

6. The physiological events in the pathway from motor-axon collaterals to motoneurones are summarized in diagrammatic form in Fig. 18.

7. The functional significance of this inhibitory pathway and of Renshaw cells is briefly discussed. A sufficient explanation is thereby provided for the inhibitory action of antidromic volleys on motoneurones. The previously postulated interaction by the electric fields which are set up by currents around active cells appears to be insignificant.

The authors wish to express grateful acknowledgement to Hoffmann-La Roche Inc. for the prostigmine bromide, acetylcholine chloride and NU 2126; to Eli Lilly and Co. for the TEPP; to Merck and Co. for the dihydro-β-erythroidine hydrobromide; and to Elliotts and Australian Drug Pty. Ltd. for the D-tubocurarine chloride.

REFERENCES

BACQ, Z. M. & BROWN, G. L. (1937). Pharmacological experiments on mammalian voluntary muscle, in relation to the theory of chemical transmission. *J. Physiol.* **89**, 45–60.

BALTHASAR, K. (1952). Morphologie der spinalen Tibialis- und Peronaeus-Kerne bei der Katze. *Arch. Psychiat. Nervenkr.* **188**, 345–378.

BARAKAN, T. H., DOWNMAN, C. B. B. & ECCLES, J. C. (1949). Electric potentials generated by antidromic volleys in quadriceps and hamstring motoneurones. *J. Neurophysiol.* **12**, 393–424.

BRADLEY, K., EASTON, D. M. & ECCLES, J. C. (1953). An investigation of primary or direct inhibition. *J. Physiol.* **122**, 474–488.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–462.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1953). Intracellular recording from antidromically activated motoneurones. *J. Physiol.* **122**, 429–461.

BROOKS, C. McC., DOWNMAN, C. B. B. & ECCLES, J. C. (1950). After-potentials and excitability of spinal motoneurones following antidromic activation. *J. Neurophysiol.* **13**, 9–38.

BROOKS, C. McC. & ECCLES, J. C. (1947). Electrical investigation of the monosynaptic pathway through the spinal cord. *J. Neurophysiol.* **10**, 251–274.

BROWN, T. G. (1914). On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. *J. Physiol.* **48**, 18–46.

CAJAL, S. R. (1909). *Histologie du Système nerveux de l'Homme et des Vertébrés*, vol. **1**. Paris: Maloine.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1953). The action of the inhibitory synaptic transmitter. *Aust. J. Sci.* **16**, 1–5.

COWAN, S. L. (1938). The action of eserine-like and curare-like substances on the responses of frog nerve-muscle preparations to repetitive stimulation. *J. Physiol.* **93**, 215–262.

DALE, H. H. (1934). Pharmacology and nerve endings. *Proc. R. Soc. Med.* **28**, 319–332.

DALE, H. H. (1952). *Transmission of effects from nerve-endings.* (Lecture) Oxford University Press.

ECCLES, J. C. (1953). *The Neurophysiological Basis of Mind: the Principles of Neurophysiology.* Oxford: Clarendon Press.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1953). Cholinergic and inhibitory synapses in a central inhibitory pathway. *Aust. J. Sci.* **16**, 50–54.

ECCLES, J. C., FATT, P. & LANDGREN, S. (1954). The 'direct' inhibitory pathway in the spinal cord. *Aust. J. Sci.* **16**, 130–134.

ECCLES, J. C., FATT, P., LANDGREN, S. & WINSBURY, G. J. (1954). Spinal cord potentials generated by volleys in the large muscle afferents. *J. Physiol.* **125**, 590–606.

ECCLES, J. C. & RALL, W. (1951). Effects induced in a monosynaptic reflex path by its activation. *J. Neurophysiol.* **14**, 353–376.

ECCLES, J. C. & SHERRINGTON, C. S. (1931). Studies on the flexor reflex. VI. Inhibition. *Proc. Roy. Soc.* B, **109**, 91–113.

FELDBERG, W. (1950). The role of acetylcholine in the central nervous system. *Brit. med. Bull.* **6**, 312–321.

FELDBERG, W. (1952). Central excitation and inhibition. *Proc. Roy. Soc.* B, **140**, 199–202.

FORBES, A., SMITH, O. C., LAMBERT, E. F., CAVENESS, W. F. & DERBYSHIRE, A. J. (1933). The central inhibitory mechanism investigated by means of antidromic impulses. *Amer. J. Physiol.* **103**, 131–142.

GESELL, R. (1940). Forces driving the respiratory act. *Science*, **91**, 229–233.

HAGBARTH, K. E. & NAESS, K. (1951). Reflex effects of tetanic stimulation of different afferent fibre-systems in the hind limb of the cat. *Acta physiol. scand.* **21**, 336–361.

HELLAUER, H. F. & UMRATH, K. (1948). Über die Aktionssubstanz der sensiblen Nerven. *Pflüg. Arch. ges. Physiol.* **249**, 619–630.

HOLMGREN, B. & MERTON, P. A. (1954). Local feedback control of motoneurones. *J. Physiol.* **123**, 47–48*P*.

HOLMSTEDT, B. & SKOGLUND, C. R. (1953). The action on spinal reflexes of dimethyl-amido-ethoxyphosphoryl cyanide, 'Tabun', a cholinesterase inhibitor. *Acta physiol. scand.* **29**, suppl. 106, pp. 410–427.

HOLTON, F. A. & HOLTON, P. (1952). The vasodilator activity of spinal roots. *J. Physiol.* **118**, 310–327.

JUNG, R. (1953). *The Spinal Cord, Ciba Foundation Symposium,* p. 130. London: Churchill.

KÖLLIKER, A. (1891). Zur feineren Anatomie des centralen Nervensystems. Zweiter Beitrag. Das Rückenmark. *Z. wiss. Zool.* **51**, 1–54.

LENHOSSEK, M. (1893). *Das feinere Bau des Nervensystems im Lichte neuester Forschungen.* Berlin: Kornfeld.

LLOYD, D. P. C. (1943). The interaction of antidromic and orthodromic volleys in a segmental spinal motor nucleus. *J. Neurophysiol.* **6**, 143–151.

LLOYD, D. P. C. (1946). Facilitation and inhibition of spinal motoneurones. *J. Neurophysiol.* **9**, 421–438.

LLOYD, D. P. C. (1949). Post-tetanic potentiation of response in monosynaptic reflex pathways of the spinal cord. *J. gen. Physiol.* **33**, 147–170.

LLOYD, D. P. C. (1951*a*). Electrical signs of impulse conduction in spinal motoneurons. *J. gen. Physiol.* **35**, 255–288.

LLOYD, D. P. C. (1951*b*). After-currents, after-potentials, excitability, and ventral root electrotonus in spinal motoneurons. *J. gen. Physiol.* **35**, 289–321.

LLOYD, D. P. C. (1952). Electrotonus in dorsal nerve roots. *Cold Spr. Harb. Symp. quant. Biol.* **17**, 203–219.

MAGOUN, H. W. & RHINES, R. (1946). An inhibitory mechanism in the bulbar reticular formation. *J. Neurophysiol.* **9**, 165–171.

NIEMER, W. T. & MAGOUN, H. W. (1947). Reticulo-spinal tracts influencing motor activity. *J. comp. Neurol.* **87**, 367–379.

RENSHAW, B. (1941). Influence of discharge of motoneurons upon excitation of neighbouring motoneurons. *J. Neurophysiol.* **4**, 167–183.

RENSHAW, B. (1942). Effects of presynaptic volleys on spread of impulses over the soma of the motoneuron. *J. Neurophysiol.* **5**, 235–243.

RENSHAW, B. (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *J. Neurophysiol.* **9**, 191–204.

SPRAGUE, J. M. (1951). Motor and propriospinal cells in the thoracic and lumbar ventral horn of the rhesus-monkey. *J. comp. Neurol.* **95**, 103–124.

STRÖM, G. (1951). Physiological significance of post-tetanic potentiation of the spinal monosynaptic reflex. *Acta physiol. scand.* **24**, 61–83.

TOENNIES, J. F. (1949). Die Erregungssteuerung im Zentralnervensystem. *Arch. Psychiat. Nervenkr.* **182**, 478–535.

TOENNIES, J. F. & JUNG, R. (1948). Über rasch wiederholte Entladungen der Motoneurone und die Hemmungsphase des Beugereflexes. *Pflüg. Arch. ges. Physiol.* **250**, 667–693.